54° Congresso Nazionale della Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica (SIBioC - Medicina di Laboratorio)

Genova, 5-7 ottobre 2022

Riassunti Sessioni Scientifiche

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SP	Sessione Plenaria
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Nota dell'Editore: i riassunti sono stati riprodotti senza alcuna revisione dal materiale direttamente fornito dagli autori.

SP01 - Sfide ed opportunità per la creazione del valore

SP01 – 01

LABORATORY MEDICINE: NOT ONLY RESILIENCE

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The COVID 19 outbreak has spread in 2020 to become the most severe pandemic in the last one hundred years. Starting as a public health crisis, it has soon become an economic crisis as well, which will have serious consequences on both individual and societal well-being, now and in the future. COVID 19 has also revealed latent health system fragilities that existed before the outbreak, highlighting the need to consider the resilience of health systems as an equally important dimension of health system performance.

Laboratory Medicine has had and continues to play a crucial role in diagnosing the Covid-19 at an early stage, recognitioning patients that need hospital care, monitoring hospitalized patients, following up patients with long-term COVID-19, and in epidemiologic surveillance.

During the Covid-19 pandemic, Laboratory Medicine is once more demonstrating its inherent and well-known resilience, that means the ability to maintain the laboratory's core mission and operations despite facing a significant crisis.

In our opinion, Laboratory professionals should however embrace a holistic approach, looking beyond developing the ability to recover or adjust to change, but rather adopting an antifragility mindset.

Antifragility is beyond resilience or robustness. The resilient resists shocks and stays the same; the antifragile evolves and gets better.

The road to becoming antifragile is fostering innovation. Innovation is about new ways of create value.

Innovation is crucial, but must be responsible.

Responsible innovation means taking care of the future through collective stewardship of innovation in the present.

Responsible innovation has always been at the heart of laboratory medicine, where we have identified three main topics areas for implementation.

The first refers to the promotion of emerging technologies; new technologies are positively associated with greater efficiency, reduced errors, and improved quality in service delivery and patient outcomes.

The second refers to the enhancement of operational excellence; operational efficiency relates to the ability of a laboratory test to provide fast and efficient results, which may improve the management of the patient with an impact in hospital efficiencies.

The third refers to the improvement of relationships at the clinical interface; laboratory professionals must interact with clinicians throughout the whole diagnostic process as part of a multidisciplinary team aiming to optimize clinical outcomes.

In fact, the key objective of laboratory medicine is to contribute in the decision-making process that ensures the best health outcome for the individual patient.

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SP02 - 01

PERFORMANCE SPECIFICATIONS IN THE IVDR ERA

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In recent decades, the awareness of the important role of laboratory Medicine for the management of status of health of patient has become increasingly prevalent and numerous scientific evidences support this thesis. It is recognized that Laboratory Medicine is a strategic centre of diagnostic medicine and the Institute of Medicine has included Laboratory Medicine services among the ten categories of essential services in the healthcare system of the United States. The reliability of the laboratory results is therefore an essential element to ensure the best management of the patient's healthcare and the evaluation and monitoring of the laboratory performances are important tools to guarantee patient safety. The new European In Vitro Diagnostic Medical Device Regulation, IVDR (EU) 2017/746, requires manufacturers of in vitro diagnostic systems (IVDs) to demonstrate, with scientific evidences, the compliance of their IVDs with the intended purpose, by developing an evaluation plan that test the scientific validity, analytical performance and clinical performance.

In this context, as defined in the Milan Strategic Conference, it is strategic the identification of suitable specifications that define the level of performance to be achieved in relation to desired outcome and comply with practicable (test and economical) requirements and can be updated with regard to the continuous innovation of Laboratory Medicine.

The implementation of a stringent evaluation process of IVDs by manufacturers and the availability of detailed information in technical data sheet, in addition to facilitate the verification of examination procedure according to the ISO 15189 requirements by laboratories, could promote a higher level of quality of laboratory results through the use and spread of suitable performance specifications.

The need of laboratory performance at more and more higher quality levels, is a requirement that cannot be ignored by laboratory professionals that, in collaboration

with the stakeholder of diagnostic process, have to define suitable performance specifications to achieve the desired outcome or reference standards, as a tool to guarantee the patient safety.

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SP01-CO01

EVALUATION OF THE K-INDEX IN INFLAMMATORY DISEASES OF THE CENTRAL NERVOUS SYSTEM IN THE PEDIATRIC PATIENT

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INTRODUCTION: the analysis of cerebrospinal fluid (CSF) and the determination of free light chains (FLC) are routinely used in the laboratory. We investigated the utility of Kappa INDEX as a specific biomarker to identify beyond pediatric multiple sclerosis (MS) also the inflammatory neurological disorders (NID), non-infiammatory neurological diseases (NNID) and other congenital neurological conditions in a pediatric population. METHOD: among 978 patients enrolled in the Clinical Biochemistry Laboratory of Turin (AOU Città della Salute e della Scienza) from January 2019 to May 2022, we analyzed 53 pediatric cases (age 0-17 years), 20 males and 33 females. Albumin, IgG and FLC Kappa in serum and liquor were measured by turbidimetric method, while isoelectrofocusing was performed by oligoclonality test. RESULTS: in according to the clinical diagnosis we obtained four different groups: MS including 6 patients (12%), NID including 25 patients (50%), NNID with 13 subjects (26%) and 6 patients (12%) considered as negative controls (NC). First, ROC curve analysis carried out comparing the SM group to all the others (NID, NNID, NC) showed that a k-index cut-off of 6.2 yielded 83.3% sensitivity and 85.7% specificity, according to data from of the latest publications (Biomolecules 2022,12,677) and the cut-off used by our laboratory (6.15) (Journal of Neuroimmunology 339, 2020). The IgG index (result ³ 0.5, 50% of sensitivity and 45.5% of specificity) confirmed the poor analytical significance. Second, the comparison between NID and NNID group showed that k-index at a threshold of 3.3 was associated with a sensitivity of 80.6% and a specificity of 76.5, and seems an excellent cut-off for the discrimination of pediatric inflammatory diseases, in accordance with the latest publications (Biomolecules 2022,12,677). The IgG index poor quality value was similar to the first study. CONCLUSIONS: in our pediatric population we observed a low number of MS diagnosis. The k-index cut off (6.15) used by our laboratory for MS is confirmed also for pediatric cohort. Moreover, this study found that a lower cut-off of k-index, 3.3, may be applied for the differential diagnosis of inflammatory processes of the central nervous system.

SP01-CO02

URINARY MICROBIAL SIGNATURE BY 16S RRNA ANALYSIS IN BLADDER CANCER PATIENTS

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The role of microbiome in the development of cancer is still unclear. In particular, the presence of microbes in the genitourinary tract raises the question of how the urinary microbiome can influence the development and progression of bladder cancer or how the presence of the tumor can impact the microbial balance of the urinary tract. The study of genitourinary-associated bacteria by sequencing of the bacterial 16S rDNA hypervariable genes using Next Generation Sequencing (NGS) provides a valuable tool in screening, risk identification, and therapeutic possibilities in bladder cancer. The aim of our work was to study the bacterial populations present in the urine samples of patients undergoing transurethral resection of bladder tumor (TURBT), in order to characterize the urinary-associated bacterial profile of these patients. The microbiome was analysed in the samples collected from patients undergoing TURBT (n=34) at our hospital. Each subject provided two urine samples: a first-morning (TURBT-FM) and a catheterized specimen (TURBT-C). First-morning sample of volunteer subjects were used as controls (HC; n=11). Bacterial 16S rRNA hypervariable regions V3-V4-V5 were sequenced using NGS Illumina platform. The sequencing data were evaluated to identify the operational taxonomic units and statistical analyses were performed using dedicated pipelines. Firstly, no significant differences in microbial profiling between TURBT-FM and TURBT-C samples were observed, suggesting that these two samples are comparable. Therefore, we compared TURBT-FM vs HC samples, finding a statistically significant b-diversity (Bray-Curtis index, p-value < 0.011) and showing a notable differences in the bacterial community profile between patients and controls. Based on Univariate analysis data, Lactobacillaceae and Bifidobacteriaceae families resulted less abundant in TURBT-FM than controls. In addition, we found a reduced abundance of Moraxellaceae family, observed up to at genus (Enhydrobacter) and species (Moraxella osloensis) levels. Further insights are needed to explore the role of these taxa in bladder cancer; however, our results suggest the impact of microbiome in the pathogenesis of bladder cancer.

SP02 - La Medicina di Laboratorio nella vaccinazione contro COVID-19

SP02 - 01

COVID-19: RESEARCH IN PREVENTIVE MEDICINE

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At the end of 2019 a new human-transmitted pathogen was isolated in China, and it was named SARS-Cov-2. The World Health Organization (WHO) on 11 March 2020 declared COVID-19 (the illness caused by SARS-Cov-2) pandemic. In Italy, the first European country affected by Sars-Cov-2, the virus made its appearance at the end of February, overwhelming in particular the northern regions of Italy and causing heavy loss of life and public health crisis. To deal with the pandemic, the centralization of health resources has put the entire National Health System in difficulty, causing postponements and delays in specialist and diagnostic visits and also impacting primary prevention interventions.
Already in the first months of 2020, the WHO had stressed how important it was to keep vaccination services active and prevent further deterioration of vaccination coverage, already stalled in the pre-pandemic era, to prevent children and public health from being threatened by other diseases, risking to cause the transition from one health crisis to another. If this is fundamental in middle-high-income countries, when it comes to low-middle-income countries it becomes dramatic, suffice it to say that the global vaccination coverage data already reported a stalemate in the pre-pandemic era (85%) for DTP 3 and measles vaccines.
World Immunization Week and European Immunization Week are occasions to rekindle the spotlight on the importance of all vaccinations because, although today the focus is mainly on those against COVID-19, it is necessary to remember that vaccines are a preventive tool against numerous infectious diseases and every day allow millions of lives to be saved.
Now, two vaccines against SARS-CoV-2 that are available in USA and Europe, are based on mRNA encoding the SARS-CoV-2 spike protein, a key target of neutralizing antibodies. mRNA vaccine technology is not totally new as it has already been studied and developed before for flu, Zika, rabies, and cytomegalovirus. This new kind of vaccines are formulated in lipid nanoparticles allowing mRNA delivery into cells and they can induce the production of the viral spike protein. mRNA is just transitorily expressed and does not interact with the genome.
The European Medicines Agency (EMA) gave also its approval of two COVID-19 vaccines that consist of an adenoviral vector containing the SARS-CoV-2 spike protein gene and one vaccine that contains the spike protein produced by recombinant DNA technology, using a baculovirus expression system in an insect cell line.
Ema approved the sixth vaccine for protecting against COVID-19 on 24 June 2022; it is an inactivated, adjuvanted vaccine. The vaccine contains whole particles of the original strain of SARS-CoV-2 that has been inactivated and two adjuvants (aluminium and cytosine phospho-guanine).
Vaccination has been shown to contribute to reducing deaths and severe illness from COVID-19, and to reduce the transmission of COVID-19.

SP02 - 02

MONITORAGGIO DELLA RISPOSTA ANTICORPALE AL VACCINO

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The immune response versus infective agents, thus including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), comprised the so-called adaptive immunity, which encompasses the generation of antibodies by B cells and cytotoxic activity by T cells, compounded by the immune memory, which has the role to contrast recurrent infections by the same pathogen. Serological testing has been conventionally defined as a diagnostic procedure used for detecting an immune response against an infectious agent, which can develop following either natural or artificial immunization. The crucial question that has emerged since the beginning of the worldwide COVID-19 vaccination campaign is whether or not laboratory monitoring of COVID-19 vaccination may be clinically useful and economically sustainable. Nonetheless, there are several reasons that contribute to justify the utility of serological monitoring after vaccination, including the opportunity (i) to know in advance whether vaccine recipients have passed a recent SARS-CoV-2 infection, (ii) to monitor the individual humoral response after vaccination, (iii) to verify the humoral response in presumably “low-responder” populations, and, last but not least, (iv) for timely detecting a fast decay of humoral protection [1]. According to the current knowledge, a timeline for serological testing could be suggested, entailing pre-vaccine measurement to precisely identify whether or not the subjects has been recently infected by SARS-CoV-2, followed by at least 2 following tests between 1 and 6 months to detect faster decline of anti-SARS-CoV-2 antibodies. As concerns the technical aspects, it may be advisable to use immunoassays capable to recognize antibodies targeting the entire trimeric spike protein, its S1 subunit, or its receptor binding domain, along with techniques that generate accurate quantitative values (1). Due to low degree of harmonization so far, an identical assay shall be used for longitudinal monitoring of antibodies values, and we finally advise against performing these measurements outside of clinical laboratories. That said, a major concern is rising. This is specifically due to the fact that the antigen and epitopes of the prototype SARS-CoV-2 lineage used for coating some immunoassays could no longer mirror the sequence of the spike protein or the

RBD of some circulating variants, such as, for example, the highly mutated Omicron lineage. Contextually, the anti-SARS-CoV-2 antibodies elicited by these highly mutated SARS-CoV-2 lineages could be no longer reliably detected by some commercial immunoassays. Therefore, along with compulsory re-evaluation and revalidation of their methods against live virus neutralization assays, diagnostic companies must also embark in redesigning assays by replacing ancestral SARS-CoV-2 antigens with those of highly mutated SARS-CoV-2 variants (2).

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SP02-03

MONITORAGGIO DELLA RISPOSTA CELLULARE AL VACCINO

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La vaccinazione per COVID-19 e l'infezione da SARS-CoV-2 inducono sia una risposta umorale mediata dagli anticorpi prodotti dai linfociti B, di tipo neutralizzante (NAbs) e non-neutralizzante, sia immunità cellulare mediata da T linfociti e cellule B di memoria. Nello sviluppo e nella sorveglianza dell'immunità a seguito di vaccinazione, la ricerca si è inizialmente focalizzata sul ruolo degli anticorpi neutralizzanti con minor interesse per il ruolo delle cellule T e B di memoria, ed ancora con scarso interesse per il ruolo degli anticorpi non-neutralizzanti che, comunque, conferiscono protezione grazie a meccanismi di opsonizzazione e citotossicità cellulare anticorpo-dipendente. Vi è, però, crescente evidenza dell'importanza del contributo delle cellule T alla risposta immunitaria dell'ospite ed in particolare per la rapida e durevole protezione rispetto l'infezione o re-infezione da SARS-CoV-2, specialmente nei confronti delle nuove varianti di interesse (VOC). Vi è sempre maggior consapevolezza che la diminuzione della protezione verso malattia asintomatica o COVID-19 di lieve entità non sia accompagnata da un declino parallelo della protezione verso malattia severa, protezione che rimane elevata -attorno al 70%- anche a distanza di mesi dal completamento del ciclo primario di vaccinazione ed anche a fronte di una significativa diminuzione degli anticorpi circolanti anti-SARS-CoV-2. Una possibile spiegazione del fenomeno proviene da un numero sempre maggiore di studi che hanno valutato l'efficacia dell'immunità cellulare anche verso linee altamente mutate di SARS-CoV-2, in particolare Omicron, che concordano nel dimostrare che la vaccinazione determina una risposta immunitaria cellulare tale da cross-reagire con una moltitudine di varianti di SARS-CoV-2, inclusa Omicron. Dal punto di vista della medicina di laboratorio, vi sono molti aspetti che devono essere affrontati, in particolare le tematiche della riproducibilità e standardizzazione dei metodi di studio dell'immunità cellulare visto che molti studi finora sono stati eseguito con metodi sperimentali e su casistiche poco rappresentative. Tuttavia, alcuni di questi metodi sono oggi disponibili a livello commerciale e devono essere verificati e validati nei laboratori clinici.

SP02-CO03

EVALUATION OF QUANTIFERON SARS-COV-2 INTERFERONG (INF G) RELEASE ASSAY IN TWO COHORT OF BNT162B VACCINATED FRAGILE PATIENTS

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Background: Severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2) vaccination is the standard of care for the prevention of COVID-19 disease, with a positive impact in countries in which vaccination has been promoted. Since the emergence of variants of concern (VOCs) European Medicines Agency (EMA) recommended an extra dose of the COVID-19 vaccines Comirnaty (BioNTech/Pfizer) and Spikevax (Moderna) for patients with severely weakened immune system and booster doses for subjects with normal immune system to ensure a lasting response. Although Vaccination triggers both humoral and cellular immune response, COVID-19 vaccination efficacy is evaluated by measuring antibodies only, whereas adaptative cellular immunity is unexplored. Our aim is to test this new kit QuantiFERON SARS-CoV-2 to evaluate the immune response after three doses of BNT162b vaccine in healthy donors compared to two cohort of fragile patients: Common

Variable Immunodeficiency (CVID) patients and Kidney Transplant Recipients (KTR) patients.
Methods: Blood samples were collected from eight health care workers in our department, fourteen CVID patients and eight KTR patients. All the individuals recruited were naïve to SARS-COV2 and immunized by three doses of BNT162b vaccine. We examined humoral responses to vaccinations using the LIAISON DIASORIN "SARS-COV-2 S1/S2 IgG". Next blood from all participants was subjected to the novel Interferon γ (INF-γ) Release Assay (IGRA) from Qiagen, measuring INF-γ release induced by two proprietary SARS-CoV-2 peptide pools (Ag1 and Ag2) encompassing the spike protein and designed to stimulate CD4+ and CD8+ T cells and induce the releases of INF-γ. Results:Using LIAISON "SARS-COV-2 S1/S2 IgG" assay from DIASORIN we confirm that in healthy subjects BNT162b third dose had successfully mounted humoral immune response with a S1/S2 IgG mean of 17100 BAU/ml. Conversely, the CVID group and KTR group shown a statistically significant reduction of IGg levels with a mean of 978 BAU/ml and 1029 respectively. Notably seven patients (five CVID and two KTR) presented IGg levels below the cut-off (33,8 BAU/ml). Next, we evaluated the INF-γ response to SARS-CoV-2 Ag1 and Ag2 founding seven non-reactive subjects (three CVID and four KTR). Surprisingly three of non-reactive patients shown a good humoral response, whereas three patients with a negative humoral immune response shown reactiveness to IGRA assay.
Conclusions: Assessing cellular immunity for SARS-COV-2 in addition to humoral immunity is important taking into account that cellular immunity plays a pivotal role against the virus and likely its variants. Some patients with weakened immune response have no correlation between humoral and cellular immunity, suggesting that the evaluation of T cell responses could be a more sensitive clinical marker of immunization. In this scenario the evaluation of cellular immunity might be informative for clinicians to identify patients more susceptible to a severe COVID-19 disease.

SP03 Telemedicina e Medicina di Laboratorio

SP0 3- 01

MACHINE LEARNING AS A TOOL FOR LABORATORY MEDICINE, TO REALIZE TRULY PERSONALIZED CARE.

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Machine learning (ML) promises to reduce errors and increase efficiency in cognitively complex tasks in every application domain where this general computational approach is proposed. Human beings stand to benefit from being able to delegate, partially or completely (with some risk or limitations), the execution of classification tasks to machines in situations where humans are either scarce or fallible resources, or both. As known, many medical specialties rely on the services of laboratory medicine (LM) to obtain responses and data that are characterized by unparalleled quality, reliability and stability (even considering imaging exams that are not dependent on operators), ranging from blood biomarkers and genetic profiles to bacterial cultures. The implementation of machine learning in this context may result in the enhancement of various classification tasks and have a variety of downstream effects on medical practice: once a patient has been defined in terms of relevant factors (such as gender, age, ethnicity, or metabolic characteristics and lifestyle), ML may assist practitioners determine whether the findings for that patient are abnormal and pathological, or normal; this can occur even based on earlier examinations of the same patient, therefore attaining the goal of personalized or precision medicine; in order to reach the ideal of the blood test as a "liquid biopsy," these methods may also assist in determining whether a particular patient is suffering from a certain disorder (whose "signature" was discovered in their tests); also, ML may assist in determining the validity of the equipment's data (1); and in determining if it is acceptable to prescribe further particular tests to the same patient from more common, faster, and less expensive procedures (such as complete blood count) (2). The potential effect of these applications is generating a lot of attention, as noted by literature reviews (3-4), which have lately attempted to account for the exponential growth in the number of articles detailing applications in the above-mentioned contexts (and others). However, the LM community must not repeat the mistakes of other communities that have been enticed by the potential of ML without assessing either the assumptions (asking whether these systems are truly accurate on cases other than those on which they were trained, i.e., whether they are robust and therefore trustworthy in the real world, in the wild); or the potential consequences (such as, for instance, the risk of increase in defensive medicine, of greater overuse and therefore overdiagnosis, as well as of the deskilling of diagnosticians and laboratory technicians (5)).The professional figure who is sometimes advocated to use these new machines and deliver the services that these will enable must therefore be able to evaluate the reliability of these systems, the appropriateness of the requests, and the plausibility and reliability of the results, for the greater patient safety and the long-term economic, social, and human sustainability of the health system into which they will be embedded.

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SP03-CO16

THE NEED OF INFORMATIC STANDARDIZATION IN THE MANAGEMENT OF LABORATORY TEST SEMANTICS

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A centralised public electronic health record system has been established in many advanced countries to ensure continuity of care. In order to enable interoperability between the different clinical data sources and to overcome their purely documentary level, the adoption of standard criteria is needed. Laboratory medicine can make a significant contribution to this goal through its mature and well-established digitisation.However, the effectiveness of IT support depends on the synergy achieved between both communities of health informaticians and laboratory staff.Since 2015, the Italian government, with DPCM 178/2015, has established the technical rules to ensure the semantic interoperability of the FSE (electronic health record), adopting the LOINC (Logical Observation Identifiers Names and Codes) coding system for laboratory examinations. The aim is to achieve unambiguous recognition of the type, method, and result description.To date, each Laboratory Information Systems (LIS) uses its own coding systems, thus making test results from different labs not comparable de facto. Translating laboratory codes into LOINC requires a deep knowledge of both coding system and local reality. Therefore, such translation can be obtained only by a multidisciplinary approach.We present two examples of this collaboration at the level of semantic interoperability, where it is most urgently needed.In early 2010s, we performed a pioneering study in Liguria Region on a subset of laboratory data obtained from HIV infected patients, to be used for regional observational studies. Starting from this limited experience we underwent a more structured project with the Veneto Region obtaining the translation of all laboratory analyses offered in that region. The lesson learned from both experiences was that a strong commitment from specialized personnel is needed and proper tools must be used to effectively monitor the evolving realities of both laboratory technology and of the code itself. Terminology management standards, as the Common Terminology Service (Version 2) and the FHIR Terminology Server, both supported by the international standards organization Health Level 7, are available and their use is strongly suggested to support this continuous updating effort.

SP03-CO17

TELE-CONSULTATION IN THE MANAGEMENT OF SYSTEMIC LIGHT CHAIN (AL) AMYLOIDOSIS: THE PAVIA AMYLOIDOSIS CENTRE EXPERIENCE.

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Light chain (AL) amyloidosis complexity requires specialized laboratories and use of highly sensitive methods to monitor the disease for hematologic (HR) and organ response (OR). After COVID-19 outbreak, access to our laboratory was limited and a tele-consultation (TM) program was built. During the first wave, all planned visits were converted to TM. When restrictions were eased, TM was proposed to patients that did at least one in- person visit. We asked to perform at a local lab: s. protein electrophoresis, s. and u. immunofixation and measurement of s. free light chains (FLC), creatinine, NT-proBNP (or BNP), troponin I, alkaline phosphatase and 24h proteinuria. Lab already connected with the Italian Society of Amyloidosis were suggested and we verified consistent use of the same FLC assay for HR. Biopsy samples can be shipped to Pavia for amyloid typing. In 2020, 369 TM were performed. In 102 (27%) this was the first evaluation. In 73 (71%) a final diagnosis was made: 18 systemic AL, 19 localized-AL, 33 wild-type transthyretin, 2 reactive AA and 1 LCDD. In 13 (13%) amyloidosis suspicion was not confirmed and 16 (15%) were already on treatment for AL and a second opinion was discussed. A TM was done in 276 (72%) patients previously seen. Of them, 214 (80%) had systemic AL with 99 (46%) on active chemotherapy [89 (41%) proceeded with therapy, 10 (4%) suspended due to sustained HR]. Seven (3%) started a new treatment due to loss of HR. In all other cases, HR was confirmed and treatment free interval was prolonged. In 2021, 109 patients performed TM: 54 (50%) systemic AL, 44 (38%) localized-AL, 5 (5%) AA, 6 (5%) monoclonal gammopathies of clinical significance. In 11 (19%) patients ongoing chemotherapy was proceeded and a new therapy was suggested due to increased FLC along with organ biomarkers in one case. In all other cases, follow-up was advised. TM allows for an integrated approach between lab and clinical management. We reached a final diagnosis in 71% of cases and we assessed HR and OR in local lab

making clinical decisions. Despite being a response to pandemic, this hub and spoke TM model proved effective in a complex disease. The use of standardized method in laboratory practice and in the clinic is crucial to the success of TM for patients' management.

SS01 - Il contributo del Laboratorio di Coagulazione nel monitoraggio delle terapie antitrombotiche: interazione Clinica-Laboratorio

SS01-01

LABORATORY ASSESSMENT OF THE ANTICOAGULANT ACTIVITY OF THE DIRECT ORAL ANTICOAGULANTS (DOACS)

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DOACs represent a class of direct-acting oral anticoagulants which, unlike vitamin K antagonists (AVK), act by inhibiting a single coagulation factor: thrombin (Dabigatran) or Factor Xa (Apixaban, Edoxaban and Rivaroxaban). These drugs have shown equal efficacy and safety compared to VKAs in the treatment and prevention of venous thromboembolism and systemic embolism in patients with non-valvular atrial fibrillation. DOACs are given at a fixed dose and therefore do not require dosage adjustments based on laboratory test results. The unnecessary dose adjustment has probably discouraged many laboratories from implementing specific tests for the assay of DOACs. In recent years, however, consensus has been increasing among experts and scientific societies on the usefulness of evaluating, by means of laboratory tests, the anticoagulant activity of DOACs in specific patient populations, usually in urgent/emergency situations. Indeed, the inter-individual variability of the levels is very high (around 70-80%) and both post-hoc analysis of the phase III clinical trials and some "real life" studies demonstrated the presence of a correlation between the DOAC level and the risk of bleeding and thrombotic complications.

The situations in which the measurement of DOAC is recommended (or at least suggested) are: 1) in presence of hemorrhagic or thrombotic complications; 2) in patients with ischemic stroke candidates for thrombolysis; 3) in case of surgical or invasive procedures; 4) if the use of antidotes is indicated; 5) in patients in whom an excess of anticoagulation is suspected; 6) in case of simultaneous use of interfering drugs; 7) in under- or overweight patients and in any case in frail/elderly subjects; 8) in case of worsening of renal/hepatic function.

Spectrometry/high-pressure liquid chromatography represent the reference tests to measure the concentrations of plasma DOAC levels, but these techniques are often not available in general laboratories and the turnaround time is not compatible with urgent/emergency situations in which the assay is recommended/suggested. In recent years, specific tests for the determination of DOAC activity have been developed, and are easily to be implemented in any laboratory, such as: diluted thrombin time, anti-FIIa activity and ecarin time (Dabigatran) and anti-FXa activity (Apixaban, Edoxaban and Rivaroxaban). These tests are simple, relatively fast, with acceptable inter-laboratory CV%, as demonstrated by the most recent EQA exercises. The results must be expressed as concentration in ng/mL. To date, the therapeutic ranges for these drugs are not known, so in the laboratory report may be indicated: 1) the expected levels at the steady state, at trough and at peak (i.e. 2 hours after the last intake), measured in patients enrolled in the phase III clinical trials or 2) a critical level, i.e. a value below which the bleeding risk can be considered negligible (<30 or < 50 ng/mL).

SS01-02

PLATELET FUNCTION TESTING CURRENT PRACTICE AMONG LABORATORIES: HOW, WHEN, WHY AND TO WHOM

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Platelets play a central role in physiological hemostasis and also in pathological thrombosis. Quantitative and/or qualitative platelet defects promote bleeding, whereas strong platelet reactivity may associate with thromboembolic complications.

The main use of platelet function tests has been traditionally to identify the potential causes of abnormal bleeding, to monitor pro-hemostatic therapy in patients with high risk of bleeding and ensure normal platelet function either prior to or during surgery. However, nowadays there is also much renewed interest in monitoring the efficacy of anti-platelet therapy and measuring platelet hyper-function or to predict thrombosis.

The current evaluation of a potential platelet defect usually involves platelet aggregation and/or measurement of granule content/release. These test are labor intensive, costly, time consuming and require a fair degree of expertise and experience to perform and interpret therefore are largely restricted to the specialized laboratory or center. Also additional expensive specialist tests are often required (flow cytometry, platelets nucleotides, Proteomics, Next Generation Sequencing).

A number of dedicated platelet function instruments that are much simpler to use and are now utilized as point-of-care (POCT) instruments have become available in last years. Some POCT instruments have been incorporated into routine clinical use and can be utilized not only as general screening tests of platelet function but as monitors of antiplatelet therapy and to potentially assess both risk of bleeding and/or thrombosis.

Given the advances in platelet function testing field new tests, mostly POCT, have been incorporated into the routine laboratory analysis. Because of the general consensus that in vivo bleeding time should be replaced, the development of reliable, sophisticated but simple to use whole blood tests that simulate in vivo hemostasis provides the ability to screen samples rapidly before

applying our existing test set and could be used as a reliable bleeding test substitute.
Many of the simpler platelet function tests could also be potentially utilized as POCT instruments to assess bleeding risk and to monitor antiaggregant therapy. Platelet function testing is therefore become increasingly utilized outside of the specialized laboratory or center.
Finally, important developments in the platelet genome and proteome are leading to advances which may have significant impact upon the diagnosis and management of patients with hemostatic and/or thrombotic defects.
In conclusion, many tests are available to assess platelet function and no one is perfect. New platelet function tests will continue to become available. Selecting the right test requires careful consideration: purpose, timeframe, available expertise and cost.

SS01-03

IL CLINICO E L'INTERPRETAZIONE DEI DATI DI LABORATORIO NEL MONITORAGGIO DELLE TERAPIE ANTITROMBOTICHE: QUALI PROBLEMATICHE, QUALI UTILIZZI?

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Not all treatments with anticoagulant drugs require monitoring. Heparin and low molecular weight heparins (LMWH) and Fondaparinux usually do not need any laboratory measurement when the drugs are administered for prevention of venous thromboembolism (VTE), or - generally in clinical practice - when administered at full doses in relation to the body weight. In contrast, vitamin K antagonists (VKAs) always require frequent blood test (INR) controls to reach and maintain over time the required anticoagulant effect. These drugs, however, are used for many decades and clinicians have learned how to monitor their anticoagulant effect. The direct-acting oral anticoagulants (DOACs), are currently the most frequently used anticoagulant drugs for the prevention and treatment of venous thromboembolism and systemic embolism in patients with non-valvular atrial fibrillation. Being given at fixed doses, these drugs do not require routine laboratory monitoring and dose adjustments. For this reason, most clinicians have so far overlooked the issue of DOACs laboratory measurement. However, in recent years consensus has been increasing among experts and scientific societies on the usefulness of laboratory tests, to assess the anticoagulant activity of DOACs in specific patient populations, especially in in urgent/emergency situations, such as in presence of hemorrhagic complications to decide on use of the now available antidotes, or to decide on use of thrombolysis in case of thrombotic complications, or when surgical or invasive procedures are needed in emergency. For the large and increasing use of DOACs in the general population, the frequency of patients in urgent/ emergency conditions or in other clinical states requiring an assessment of their anticoagulant activity will increase sharply. Subsequently, the number of clinicians who must cope with this issue will also increase. However, several obstacles are limiting their full participation and comprehension to this activity. The decision on which tests should be available is laboratory staff's concern; however, the turnaround time of tests should be compatible with the clinical needs. Though therapeutic intervals of DOACs are not available, intervals suggested as appropriate in relation to the different clinical condition should be clearly mentioned in laboratory reports, possibly as results of collaborative decisions. Some laboratory-specific aspects, that are important for the correct interpretation of laboratory results, should be clearly detailed in written and repeatedly circulated among clinicians and nurses (this is the case -among others - for the correct timing of blood sampling in relation to LMWH or DOAC administration, or for the interference of LMWH on activity results of anti-Xa DOACs).

SS01-CO04

EVALUATION OF HYPERCOAGULABILITY IN PATIENTS WITH MULTIPLE MYELOMA USING EXPERIMENTAL PARAMETERS OF ROTATIONAL THROMBOELASTOMETRY: MAXV, MAXVT, AUC

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INTRODUCTION: Haematological malignancies are, generally, associated with an increased thrombotic risk with a thrombosis rate 28 times higher than in healthy individuals. Multiple Myeloma (MM) is a haematological neoplastic pathology with strong thromboembolic repercussions. Thrombogenicity of MM is multifactorial and risk factors are traditionally divided into three groups: patient-related clinical risk, disease-related risk, and treatment-related risk. Venous thromboembolism in these patients occurs during the first months of treatment with immunomodulatory drugs (IMiDs) in combination with high-dose dexamethasone. Although therapy with IMiDs improves the clinical outcome, it is highlighted a concomitant increase of thromboembolic manifestations which make the anticoagulant therapy in MM patients difficult to manage. To date, the viscoelastic tests, assessing global haemostasis, allow to stratify the thrombotic risk in these patients. To evaluate the coagulation dynamics proprieties, we investigated experimental parameters [Maximum Velocity of Clot Formation (MAXV), Time to MaxV (MAXVt) and Area Under the Curve (AUC)], by using Rotational

Thromboelastometry (ROTEM).
METHODS AND RESULTS: The study included 35 MM patients (at diagnosis and follow-up) and 35 healthy subjects unrolled from Haematology Division of AOU Federico II of Naples. The EXTEM, INTEM and FIBTEM assays were overall investigated for both standard and experimental parameters. Particularly, among the experimental parameters, evaluating coagulation dynamics proprieties, we observed statistical significance differences between controls and patients for EXTEM (MAXV: p=0.003; MAXVt: p=0.01), for INTEM (MAXV: p=0.005; MAXVt: p=0.043) and for FIBTEM (MAXV: p=0.001; AUC: p=0.0005) tests.
CONCLUSION: To the best of our knowledge, this is an original study showing that these experimental parameters can give an additional contribution in the interpretation of the hypercoagulability state, both at diagnosis and at follow-up. This approach could be a valid tool for a prompt response of the coagulative status, compared to numerous and more investigating classic coagulation tests and allowing physicians to give a timely and adequate anticoagulant treatment.

SS01-CO05
FACTOR V DEFICIENCY REVEALED DURING DOAC THERAPY

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A 68-years-old-man, with a history of bleeding diathesis not investigated, after an adenoidectotmy and a post traumatic splenectomy, accessed to the first aid of the Pontedera Hospital for intense asthenia and discharge of black stools. Laboratory tests revealed severe anemia (hemoglobin 4 g/dL), Prothrombin Time (PT) 3,42, activated Partial Thromboplastin Time (aPTT) 3,8. In the previous months onset of exertional dyspnea, lower extremity edema and diagnosis of atrial fibrillation. It was administered to him low molecular weight heparin (LMWH) replaced later with dabigatran 150 mg BID to prevent stroke. Dabigatran was later suspended due to gastric intolerance and replaced with edoxaban
60 mg SID. The colleagues from the Pontedera Hospital suspended edoxaban and request second level coagulation tests, they sent us frozen citrated plasma samples. We first made sure there was no residual edoxaban in the circulation. Then we did the mixing tests for PT and aPTT, at room temperature and after incubation for two hours at 37°C. The mixing tests corrected coagulation times. Measures of coagulation factors revealed us severe deficiency of FV (1,1%). The other factors resulted: FVII 41%, FXII 51%, FIX 117%, FXI 107%, FVIII 171%, FX 65%, FII 62%. No antibodies against FV, FII and FVII were found. The patient was sent to Antithrombosis Centre in Florence to evaluate the most appropriate therapy to prevent stroke and the opportunity of the surgical closure of the left atrial appendage.Conclusions- When starting anticoagulant treatment, according to consolidated guidelines, knowledge baseline blood works (blood count, renal and liver function, full coagulation panel) is required. Our patient, which showed a likely congenital deficiency of FV, was treated with DOAC without having performed recommended laboratory test and his bleeding diathesis after surgery was probably not carefully evaluated. The collaboration between colleagues with different skills and experience is the most effective way to diagnose rare disease and to choice the most appropriate therapy in a comorbidity setting.

SS02 - Ruolo del monitoraggio terapeutico del farmaco nella moderna Medicina di precisione: considerazioni tra presente e futuro

SS02-01
IL MONITORAGGIO TERAPEUTICO DEL FARMACO (TDM): CRITICITÀ IN ETÀ PEDIATRICA

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A recent study published by Divya Hoon and colleagues (2019) has shown that in 44.5% of visits to office-based physicians who prescribed systemic drugs to children, these drugs were prescribed off-label (1). The term "off-label" use refers to use of a drug that is not included in the package insert (approved labeling) for that medication. Specifically, dugs are used off-label when administered for an unapproved indication or in an unapproved age group, dosage, or route of administration (2). Unfortunately, the majority of drugs administered to neonates and children are off-label due to the lack of clinical studies conducted on this special population. As consequence, dosing strategies adopted to treat children are often translated from adult studies by using allometric scales. However, pharmacological treatments applied to these patients should deserve more tailored dosing approaches. Therapeutic Drug Monitoring (TDM) is defined as assessing the adequacy of the drug plasma concentrations in relation to a target concentration or concentration window at a specific time in a dosing interval. Therefore, TDM represents an useful tool not only for monitoring plasma concentrations of drugs with a narrow therapeutic index but also to evaluate the PK behavior of different medications especially when administered to neonates

and children. Unfortunately, TDM-based studies are not always applicable to these patients due to ethical and physiological concerns that limit the number of samplings and the volume of blood withdrawable. In this brief communication, we will focus on the main criticisms in performing TDM in neonatal and pediatric patients. These will include:

- Age-related pharmacokinetic (PK) variability. In fact, developmental and physiological changes that characterize neonates and children can dramatically affect PK behavior of many drugs (3);
- Necessity of sensitive bioanalytical technologies. TDM should rely on analytical methods such as high-performance liquid chromatography coupled to UV (HPLC-UV) or to mass spectrometry (LC-MS/MS) characterized by fast detection, high accuracy and precision (4);
- Novel microsampling strategies that are not influenced by heamtocrit (Hct). Volumetric absorptive microsampling (VAMS) have been explored as alternative to dried blood spot (DBS) and allow to overcome the DBS-associated Hct effect (5);
- Need for therapeutic ranges tailored on neonates and children. Children are not small adults and evidence-based therapeutic ranges are arguably even more important in these subjects;
- The impact of excipients in pediatric formulations. Excipients such as propylene glycol should be closely monitored alongside to drugs' levels for their possible toxicity.
- The risk of drug-drug interactions during poly-therapy. TDM is particularly advisable when multiple drugs are administered in order to monitor PK interactions;
- The occurrence of metabolism-related adverse events. Some pharmacological treatments can interact with specific metabolic pathways leading to adverse reactions (i.e hyperammonemia).

Therefore, the aim of this presentation will be not only to describe these criticisms but also to provide possible suggestions to overcome them.

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SS02-02

PERSONALIZZAZIONE DELLA TERAPIA NEL PAZIENTE ADULTO E ANZIANO.

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Pharmacogenetics (PG) represents the "branch" of pharmacology that guides the personalization of pharmacological therapies. Alongside PG, Therapeutic Drug Monitoring (TDM) has been proposed and included in many different guidelines for numerous therapies in order to optimize the treatment regimen and personalize therapies in young, adult and elderly patients. The TDM of drugs is used daily in various clinical settings where it represents some help to reach a further possibility of clinical-therapeutic success, especially in particular clinical cases and setting, promptly personalizing the therapy. The elderly patient, specifically, has physiological characteristics (lower renal clearance, lower hepatic metabolism, etc.) capable of making the pharmacokinetics of the drugs substantially unpredictable. Moreover, in the elderly patient often could be present the more consistent problem of polytherapy due to the concomitant presence of various pathologies to be treated (as diabetes, hypertension, hypercholesterolemia, etc.). Other than the "small molecules", many of the new biological drugs, such as monoclonal antibodies, may also show different pharmacokinetic patterns depending on the patient's age. Therefore, both the methodological aspect of TDM, both the pharmacological profile obtained from it, and the rationale for its use, are extremely relevant for obtaining reliable results and, at the same time, helping the clinician in diagnostic and therapeutic decisions, with the final aim to personalize the therapies.

SS02-03

ANTIVIRAL DRUGS FOR THE TREATMENT OF COVID-19 PATIENTS: APPLICATIONS OF DRUG MONITORING

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In the last years, several studies on new and/or repurposing antiviral drugs were initiated to fight Coronavirus disease 2019 (COVID-19) pandemic. Three antivirals have so far been authorised in Italy for the treatment of COVID-19 in adults who do not need supplemental oxygen and

who are at high risk of progressing to severe COVID-19. Specifically, the drugs currently authorized are Remdesivir (intravenous route) and the orally administered Molnupiravir and Nirmatrelvir-Ritonavir (Paxlovid). Remdesivir is a prodrug of the nucleotide analogue (GS-441524) which inhibits the SARS-CoV-2 RNA-dependent RNA polymerase. Remdesivir has been shown to improve the COVID-19 outcome in different settings. Molnupiravir is a prodrug that after entering in the cells changes into an active form of triphosphate, ready to be incorporated into viral genome causing many errors in SARS-CoV-2 RNA. In a clinical trial, Molnupiravir compared to placebo showed a 30% reduction of COVID-19-related hospitalizations. Paxlovid is a combination of Nirmatrelvir and Ritonavir. Nirmatrelvir acts by inhibiting protease enzyme, essential step to transform some viral proteins into their final functional form. The relative risk reduction of hospitalization or all-cause death at day 28 for Paxlovid compared to placebo was 88%. To guarantee safe and effectiveness of the pharmacological therapies, the evaluation of patient's pharmacokinetics (PK) profile is mandatory. Therefore, the monitoring of drug concentration of antivirals against SARS-CoV2 could be pivotal to optimise drug regimens, increase efficacy and avoid drug-related toxicity and to evaluate intra-individual variability and drug-drug interactions. Actually, few data on antiviral drugs concentrations in COVID-19 subjects are published. Among them, the most interesting are the following: i) Remdesivir and its main metabolite showed high PK interpatient variability due to both age and renal function in COVID-19 inpatients. The PK variability may have a potential effect in determining the efficacy of Remdesivir administration in patients affected by COVID-19. ii) Molnupiravir metabolite penetration into upper airways and mucosal secretions was demostred. These data could support the use of molnupiravir in a prophylaxis for SARS-CoV-2 infection iii) Nirmatrelvir displays a short half-life, which could result in suboptimal drug exposure and difficulties in achieving efficacy. Therefore, there is the need to use ritonavir (CYP3A4 Inhibitor) to slow down the metabolism and to increase the plasma concentrations of Nirmetrelvir. Drug-drug interactions are expected when drugs metabolized by CYP3A4 are co-administered with Paxlovid. Further research on antiviral drugs concentrations in COVID-19 patients could help to define therapeutic strategies more efficient and appropriate to treat SARS-CoV-2 infection.

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SS02-04

THERAPEUTIC DRUG MONITORING IN ALTERNATIVE MATRICES: FROM LABORATORY TO CLINICAL PRACTICE

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Therapeutic Drug Monitoring (TDM) is a diagnostic tool used for years in daily clinical practice to optimize the exposure of patients to drugs, with the goal to maximize the therapeutic response while preventing at the same time the development of drug-induced toxicity.

TDM consists essentially in determining the concentrations of a drug in an easily accessible biological matrix, normally serum, plasma or whole blood obtained from venipuncture, on the primary assumption that systemic drug concentration is reflective of that attained in the target site. This information is then used to individualize dosage so that drug concentrations can be maintained within a therapeutic target range.

Since blood remains the gold standard for TDM, the majority of the therapeutic target ranges refer to this biological matrix. However, alternative matrices could provide complementary information and more often clinicians request drug determination in matrices other than plasma, as detailed below

The use of saliva as an alternative to conventional matrices has been a subject of interest for decades and has been proposed for several drugs. The main advantages of saliva are the ease of collection not requiring invasive procedures, the limited (if any) purification steps and the reduced costs. It has been proposed that salivary levels can represent a surrogate marker of the free drug concentration in the plasma (also referred as protein-unbound fraction), which is the active drug fraction responsible of the pharmacological effect. Actually, the correlation between saliva and plasma drug levels depends on many variables such as molecular weight and the negative log of the acid dissociation constant (pKa) of the compound, as well as its lipophilicity and the ionization status. In addition, the procedure of sample collection, the oral flow rate and the possible oral contamination, are key variables known to

impact on the variability of salivary drug concentration. All together, these factors have largely hampered the use of this matrix, limiting the application of saliva-based TDM to a limited number of analytes (mostly antiepileptic drugs) and not in clinics as a diagnostic tool.

Conversely interest has grown for the TDM using alternative biological matrices in special population, as in the case of breastfeeding women. Currently, nearly 90% of the pregnant women start breastfeeding after partum, and more than half of them take medications. This implies that a large number of breastfed infants are potentially exposed to medications in uterus and in human milk from breastfeeding women. While most of the drugs that women take during pregnancy or post-partum are not associated with known adverse outcomes of the infant, several case reports of serious infant toxicity have been published in literature reporting the importance of the determination of drug content in amniotic fluid and/or in breast milk. This atypical TDM can help physicians to evaluate the exposure of the fetus or infant to the drug, eventually establishing a causal effect with the development of the adverse event. In fact, during pregnancy, drug concentrations in fetal serum at steady state are in equilibrium with the maternal serum drug concentrations that, in turn, are governed by maternal dose per time and clearance. By contrast, infant drug exposures through breast milk are defined by infant dose via milk (per time) and infant drug clearance. For each mother–infant pair, it is, therefore, important to identify factors that can increase the excretion of the drug into breast milk (as decreased maternal clearance, maternal overdosing) or decreased the infant drug clearance (i.e. liver or kidney disease, prematurity). In fact, both these conditions may theoretically increase the infant serum drug concentration, and thus increase the risk of the adverse drug reaction(s).

In our center, the TDM requests for determination of breast milk drug concentrations mainly deal with drugs active on the central nervous system such as antiepileptic drugs, antidepressants and/or antipsychotics.

Another special population that may benefit from the TDM in alternative matrices is represented by critically ill patient treated with anti-infective drugs. Indeed, for these drugs, it is mandatory to quantify the amount of drug that reaches the site of infection and to correlate it with the microbiological data (i.e. the minimum inhibitory concentration, MIC). If the concentrations of the drug in the site of infection are higher than the MIC, there is a high probability of success of the antimicrobial therapy. For these drugs, the measurement of systemic concentrations is poorly predictive of antimicrobial response due to anticipated differences in distribution of antibiotics or antifungal to the tissue compartments.

Bronchoalveolar lavage (BAL) is a semi-invasive method used in both research and clinical practice as a way of quantifying drug concentrations from epithelial lining fluid (ELF) and/or alveolar cells (AC) in pulmonary infections. The drug concentration ratio between ELF or AC and plasma, is important to guarantee that sufficient drug concentrations reach the pulmonary tract.

Currently, there are a limited number of BAL studies measuring ELF concentrations in critically ill patients due to practical and ethical issues. However, in selected clinical situation, such as ventilator-associated bacterial pneumonia, where pathogens may have elevated MIC values, or in the case of patients with modified plasma protein content, the availability of BAL concentration can be important for optimizing dosage regimens of antibacterial agents.

In the last few years, the TDM of drug concentration in tissues has gained interest and popularity, because it can provide better correlation to therapeutic effect. This is the case also for immunosuppressive drugs. In fact, evidence suggests that intra-graft and intracellular concentrations may, more accurately, predict the outcomes of transplant recipients as it may provide a better understanding of drug distribution during graft rejection.

Unlike liquid matrices, bioanalysis in tissues offers unique challenges such as proper tissue sampling, appropriate tissue sample preparation, efficient extraction of the analytes from the tissue homogenates, and demonstration of stability and recovery of analytes in intact tissues. Therefore, apart some very restricted cases, the TDM in tissue is far from becoming a routine clinical practice.

A more easily available biological matrix is represented by the cells present in blood sample such as peripheral blood mononuclear cells (PBMC), lymphocytes and monocytes, isolated, counted and lysed for the determination of intracellular drug concentration

For antiretrovirals, immunosuppressants and antileukemic drugs, information about the intracellular concentrations and intracellular distribution has been proposed as innovative markers of therapeutic drug efficacy compared with the traditional blood-based TDM.

To date, all the intracellular assays described in literature do not discriminate between drug localized into cell membranes or into the cytoplasm, whether bound to intracellular proteins or unbound, which should be the effective drug moiety. Measurement of total cell concentrations is thus of limited value, but it is the only data available at this time. In addition, several technical challenge are still a problem for the spread in use of intracellular concentration as a diagnostic tool.

Remarkably, preliminary experiences have documented that, despite the above mentioned methodological limitations, this innovative TDM can predict the response of patients to pharmacological therapies.

LC-MS/MS is recognized as reference technique for determination of drug concentrations of low-molecular-weight molecules. The high degree of precision and accuracy makes this method suitable for measurements also in human alternative matrices even in case of very low concentrations. Recent instrumental developments of sample preparation techniques have enable faster and less complicated sample pre-treatment procedures. However, LC–MS/MS is not a gold standard procedure per se, independently of the efforts given during the development and validation steps.

Matrix effect represents a key factor in method development as ion suppression or enhancement negatively influence the accuracy and sensitivity of the method. The use of isotopic internal standards and

chromatographic separation of analytes from region of ion enhancement or suppression can take under control the effect of matrix effect.
In any case, international guidelines for bioanalytical method validation advise to validate the method with the same matrix of patient sample, in order to achieve reliable results that are necessary for proper decisions on drug dosing and patient safety.
Due to the relative difficulty in finding rare blank matrices, surrogate matrices may be acceptable for analytical method validation such as a suitable buffer medium, diluted plasma or water with addition of sample proteins. It should be kept in mind that if a modification in matrix specie of a fully validated method is introduced, a new validation of the method will be necessary. In addition, although every attempt is made to formulate standards and quality controls to be as similar as possible to the specimens to be analyzed, in vitro samples can significantly differ from patient samples and incurred sample reanalysis of patient samples is advocated.
The request from clinicians for the evaluation of drug concentration in "exotic" matrices is growing in particular settings, therefore the applicability of bioanalytical methods to different matrices from plasma and serum will become an important challenge in which the laboratory will have an important role.

SS02-CO06

THERAPEUTIC MONITORING AND PHARMACOKINETICS STUDY IN LC / MS-MS OF ELEXACAFTOR / TEZACAFTOR / IVACAFTOR, DURING THE PREGNANCY PERIOD

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Cystic Fibrosis (CF) is an autosomal recessive genetic disease due to mutations of cystic fibrosis transmembrane conductance regulator (CFTR) gene, which causes a deficiency in the protein channel responsible for chlorine transport, making secretions thicker causing severe damage to the lungs, digestive system, and other organs. Currently more than 2000 mutations have been identified of which the most frequently encountered in affected patients is the deletion of phenylalanine at position 508 (F508del). In 2020, a new drug called "Kaftrio" was approved by EMA for the treatment of CF patients from the age of 12 and carrying at least one F508del mutation, given by the combination of three molecules (Ivacaftor, Tezacaftor and Elexacaftor (ETI)) capable of correcting the malfunction of the mutated protein by partially restoring its activity. The improvement in clinical conditions and life expectancy, associated with an improvement in female fertility due to greater fluidity of the cervico-vaginal mucus, leads to an increasing number of women with CF to become pregnant. The Infectious and Tropical Diseases department and the Regional Reference Center for Perinatal Toxicology of the Careggi University Hospital of Florence requested the support of the General Laboratory to carry out the therapeutic monitoring and study of the pharmacokinetics of ETI in a 33-year-old patient suffering from CF during the pregnancy. This request derives from the lack of exhaustive guidelines and data in the literature that report the changes in the concentration of the active principles and its metabolites during the gestational period. In our laboratory, a quantitative method in LC / MS-MS was optimized and validated using 4000 QTrap mass spectrometer (ABSciex) for the therapeutic monitoring of the various analytes on plasma samples at 0h, 2h, 4h, 6h, 8h and 24h at each trimester of pregnancy. Thanks to the success of the results obtained, it was decided to extend the determination of these substances in a multicentre study involving women with cystic fibrosis during the gestational period to broaden the series and the assessment of drug concentrations, as well as in maternal blood, including in cord blood and breast milk.

SS03 - SIBioC Young Scientist Fight against COVID19

SS03-01

COVID-19: AN OPEN CHALLENGE BETWEEN RESEARCH AND INNOVATION

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Since the SARS-CoV-2 was isolated and identified as COVID-19 etiological agent, the traditional laboratory routine is dramatically disrupted. An enormous number of naso-pharyngeal swabs (NPS) has been tested every day for diagnosing COVID-19 and contact tracing. Although the NPS molecular testing immediately appeared to be the gold standard for diagnosing acute SARS-CoV-2 infections, it requires skilled personnel to collect NPS and performing tests, dedicated instrumentation and time to release results. In this scenario, laboratories and manufacturers worked a lot to search new and fast solutions to overcome these limitations. Saliva was proposed as a valid alternative to NPS, as its collection is easy, standardized if devices commercially available were used, independently obtained and well tolerated by subjects. Moreover, it was also demonstrated that sensitivity and specificity of both molecular and antigen testing on saliva was comparable to those obtained in NPS (1,2). These findings allow to save resources involved in sample collection and adopting saliva in active surveillance programs proposed as strategy to

limit virus spread (3). Process optimization was also object of research and innovation. New collection devices to catch SARS-CoV-2 from the subjects and environment were also developed to reduce at minimum samples handling before testing and risks for staff in the working area, especially in case of high drops dissemination (e.g. dentists). Different inactivation solutions (by liquid and lyophilized chemical solutions and heating) were also evaluated to limit contagion risk and ensure results accuracy and short turnaround time (TAT). Inactivation by heating seems to be the most effective strategy to reach the above cited aims (4). In addition to molecular testing platforms, a variety of antigen testing for SARS-CoV-2 detection (POCT and laboratory-based immunoassays) were also evaluated to identify the suitable, effective and affordable system. Lumipulse G-SARS-CoV-2 Ag CLEIA testing represents the best compromise between molecular testing and POCT, as it showed high sensitivity and specificity in NPS as well as in saliva samples, short TAT, did not require skilled personnel (1,5). These findings are very important in the current scenario. Vaccination has been demonstrated effective in reducing hospitalizations, but the number of daily infected subjects is still high due to the dissemination of highly mutated and more transmissible SARS-CoV-2 variants. Then, the continuous search for processes optimization and solutions to develop sustainable large scale screening programs remain a priority for economically activities opening and protection of fragile subjects. The prediction of host response to infection remains still difficult to understand, then the search of new biomarkers also is another interest and opened field. Proteomics in saliva appeared a promising tool for screening markers of disease occurrence and progression.

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SS03-S02

THE CLINICAL LABORATORY IN COVID-19 MANAGEMENT

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The outbreak of the COVID-19 pandemic declared worldwide1 due to the spread of SARS-CoV-2 has further highlighted the key role of Laboratory Medicine in the healthcare process. In this context, clinical laboratories needed to quickly reorganize their internal workflow to deal with the emergency and continue to ensure rapid and accurate responses. This was possible thanks to the contribution of all medical laboratory professionals, including administrative staff, nurses, clinical laboratory technician, pathologists and physicians who worked in synergy for an efficient and successful management of the COVID-19 pandemic in clinical laboratory setting. There are many activities in which clinical laboratories have been involved, not only concerning diagnostics.
In our Metropolitan Area of Bologna, the LUM (Laboratorio Unico Metropolitano) started to provide the diagnostic for SARS-CoV-2 detection since November 2020. Several diagnostic lines have been implemented, particularly those involving the detection of SARS-CoV-2 RNA and antigen by nasopharyngeal and salivary swabs. These methods have been made available in the context of urgency and routine for different types of users (inpatients and outpatients' diagnosis, healthcare workers and school screening, etc.). Results reporting also included extensive consultancy activities provided by laboratory professionals to internist, infectious disease specialists, public health departments staff. In addition to the activity of SARS-CoV-2 detection, our laboratory provided other emergency exams 24/7 from the beginning of pandemic, including the possibility to request specific inflammatory biomarkers such as serum IL-6. Moreover, through the outpatient service of our department the vaccination service has been implemented, especially for fragile and allergic subjects. In relation to this process, surveillance through dosing anti-SARS-CoV-2 antibodies was carried out; this activity allowed us to understand the dynamics of antibody response of a specific group of patients. At last, our laboratory participates in the regional and national surveillance program for detection of SARS-CoV-2 variants by sending positive samples to the regional reference laboratory. All these activities performed by clinical laboratories for the management of COVID-19 pandemic are constantly evolving and still requires effort, particularly for technological and organizational updating actions.

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SS03-03

IL RISCHIO DI REINFEZIONE DA SARS-COV-2 E LE IMPLICAZIONI PER LA DIAGNOSTICA

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One of the most interesting aspects of the COVID-19 pandemic is that a variable percentage of patients (from 2% to 69%) could have a repeated positivity following hospital discharge or even several weeks after clinical recovery (1). There are multiple reasons why a positive result to SARS-CoV-2, usually ascertained by RT-PCR, may be detected again, including reinfection, disease reactivation, prolonged viral shedding or false positive results (1-2). Since the beginning of the pandemic, several authors have reported the possibility of reinfection by SARS-CoV-2 or reactivation of a latent infection, calling for urgent attention from researchers, as well as public health policymakers. In our study we estimate incidence rate of 3.5% of reinfection in the Province of Modena in the first 6 months of 2021. Reinfection rates according to vaccinated or non-vaccinated subjects were 0.6% vs 1.1% ($p<0.0001$).

Multiple questions regarding reinfection associated with SARS-CoV-2 are still ongoing. What is the pathophysiological mechanism for reinfection? Who are the subjects with a higher risk of reinfection? What is the clinical burden for reinfected patients? Reinfection with the SARS-CoV-2 virus can be mainly attributed to two phenomena: decay of the immune response and viral mutations that favor the appearance of new variants (3-5). Currently, there are discordant rates of reinfection reported in SRs (ranging from 0-50%), which could partially be explained by the heterogeneous adopted definitions of reinfection. Today, there is still no universal agreement on the determination of the correct time period between positive results for SARS-CoV-2 for the definition of reinfection, although the definition provided by CDC is the most accredited (https://www.cdc.gov/coronavirus/2019-ncov/php/invest-criteria.html).

It has been pointed out that the severity of reinfection depends on the individual immune response, as well as both the viral load and the SARS-CoV-2 variants causing the reinfection. New virus variants could evade immune responses acquired in subjects with infections from previous variants or reduce the capacity for neutralization by polyclonal antibodies (4). This issue suggests the need to increase the current knowledge about the degree of protection provided against SARS-CoV-2, leading the development of vaccines and the creation and implementation of appropriate interventional strategies.

Because COVID-19 is a relatively new disease, several aspects of its progression and long-term health effects are unknown, one of the aspects that have become more relevant as time goes by is the impact that reinfections. There is a real, albeit rare risk of SARS-CoV-2 reinfection. Nevertheless, a standardized approach to identify and report reinfection cases should be developed.

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SS03-CO07

COMPARATIVE ASSESSMENT OF MAGLUMI SARS-COV-2AG TEST AND NUCLEIC ACID APMPLIFICATION TEST (NAAT): FIRST CONSIDERATIONS

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Introduction: NAAT is still the international reference assay for the diagnosis of Covid-19 due to high sensitivity and specificity; it is able to detect the pathogen even at low viral load, nevertheless a positive PCR result demonstrates the presence of nucleic acid in the sample, but not if it contains an infecting virus; the risk of unnecessarily isolate a person who is no longer infectious and a long TAT can preclude a screening utility. Conversely, immunoassays targeted to detect viral antigens have the potential to fit the requirements for a screening population test. In this study, we evaluated the consistency of results obtained by Maglumi SARS-CoV-2 Ag test in comparison with molecular test. Methods: 79 positive NAAT nasopharyngeal swabs (NPS) with Ct-threshold cycle (Ct) between 39 and 13 were selected and analysed with MAGLUMI CLIA assay targeted to nucleocapsid SARS-Cov-2 antigen (M) by Snibe Diagnostic. 37 NPS were also analysed with Liaijson XL (L), DiaSorin s.p.a., which is routinary used in our lab. 5 negative NPS were included in the experiment as negative controls. Results: all 5 negative NPS were confirmed negative with both CLIA assays. 33 of 79 (41,8%) positive NPS were confirmed positive on M, all these samples had a $Ct < 26$. The remaining 46 positive NPS, resulting negative on M, had a $Ct > 24$. Results for 37 NPS tested both with M and L agreed 100%. Conclusions: CLIA test demonstrated to be less sensitive to the viral presence

than NAAT, in particular M test seems to be able to detect a positivity to SARS-Cov-2 when the viral load is detected at a CT < 24. The grey area, where NAAT and CLIA were not in perfect agreement, seems to be Ct= 24 / 25. In this area M missclassified 67% of positive NPS, but further investigation with a larger number of samples is necessary to confirm this finding. While PCR positivity may persist for several weeks after the onset of the disease and the disappearance of symptoms, Ag tests reach satisfactory sensitivities when infected people are more likely to be contagious; together with the high throughput of the technology, makes them an extremely useful tool for screening population, especially during the pandemic.

SS03-CO08

QUALITATIVE AND QUANTITATIVE PERFORMANCE OF SARS- COV-2 NUCLEIC ACID DETECTION TESTS: RESULTS FROM THE 2021 EXTERNAL QUALITY ASSESSMENT IN LOMBARDY

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Introduction. Nowadays, the nucleic acid amplification tests, such as real time reverse transcription polymerase chain reaction (rRT-PCR) assays, are the gold-standard for detecting SARS-CoV-2. This study aimed to evaluate the qualitative test performance and inter-assay variations of SARS-CoV-2 nucleic acid detection tests by analysing the results of the External Quality Assessment (EQA) programme carried out in Lombardy in 2021.
Methods. 2021 EQA for SARS-CoV-2 molecular test consisted of 12 samples (cells culture supernatants): 5 SARS-CoV-2-negative and 7 SARS-CoV-2-positive (3 had viral load (VL)>5x103copies/ml, 4 VL=1-5x103copies/ ml). Each participating laboratory had to provide qualitative (i.e. positive/negative) and quantitative (i.e. Ct value) results obtained with each SARS-CoV-2 molecular test in use. Qualitative test performance was evaluated by positive/negative percent agreement (PPA/ NPA); inter- assay variation of quantitative results was evaluated by coefficient of variation (CV).
Results.79-90 laboratories participated to 2021 EQA for SARS-CoV-2 RNA detection by using 199-231 systems and returning a total of 2,623 results. PPA ranged between 99.5% (samples 1 and 7) and 100%, NPA between 69.9% (sample 4) and 100%. This latter range became 99.1-100% by ruling out the “invalid” results (n=89) due to low amount of human cells in two samples. Considering all systems, CV ranged between 10.8% and 13% for SARS-CoV-2-positive samples with VL=1-5x103copies/ml and between 11.9% and 14.2% for samples with high VL. We report here only the results of the two most used systems: system A (275 results) and B (245 results). For samples with VL=1-5x103copies/ml, CV ranges by viral target (vt) were: vt1) 6.4-9%; vt2) 6.6-7.8%; vt3) 2.5-4.3%; vt4) 2.4-3.8%. For samples with high VL, CV ranges were: vt1) 7.3-11.9%; vt2) 5.8-10.4%, vt3) 2.1-4.4%; vt4) 2.8-4.2%.
Discussion. Within 2021 EQA for SARS-CoV-2 molecular test, PPA and NPA were above 99.1%. However, the inter-assay variability among systems was notable and related both to VL and to viral target detected by the test. This variability - thought limited - needs to be carefully evaluated and continuously monitored within quality assurance programs.

SS04 - La stratificazione del rischio cardiovascolare: una sfida fondamentale per la Medicina di Laboratorio

SS04-01

CARDIOVASCULAR RISK EVALUATION IN THE GENERAL POPULATION: THEORETICAL CONSIDERATIONS, EXPERIMENTAL EVIDENCE AND CLINICAL RELEVANCE.

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Only during the last 10 years, the set-up of some immunoassay methods with high-sensitivity analytical performance (limit of detection between 1 and 3 ng/L) allowed a reliable determination of circulating levels of cardiac troponin I (cTnI) and T (cTnT) in the most part of healthy adult subjects, in accordance with quality specifications required by the most recent international guidelines. Several recent studies reported that circulating levels of hs-cTnI and hs-cTnT in healthy adult subjects show considerably lower intra-individual (from 4% to 12%) than inter-individual variations (about 50%) (1). Accordingly, the high-sensitivity methods for cTnI (hs-cTnI) and cTnT (hs-cTnT) should be considered an accurate estimate of the physiological myocardial renewal in healthy adult subjects (2). In particular, some experimental and clinical studies suggested that the 99th percentile value of biomarker distribution in the reference healthy population (i.e., 99th URL value) corresponds to the amount of hs-cTn contained in about 40 mg of myocardial tissue (2). Several experimental and clinical studies have recently demonstrated that cardio-specific biomarkers (such as cardiac natriuretic peptides and cardiac troponins) may help in the identification of apparently healthy subjects, who are at risk for accelerated progression towards symptomatic heart failure (3,4). Indeed, the cardiovascular risk progressively increases in the general population even for hs-cTnI and hs-cTnT values below the 99th percentile URL (i.e., the recommended cut-off for the detection of myocardial injury and diagnosis of myocardial infarction)

(3,4). In particular, the MORGAM/BiomarCaRe study investigated whether the change in 3 repeated measures of hs-cTnI collected 5 years apart improves 10-year prediction of cardiovascular risk in 3875 participants, aged 30–60 years at enrolment (51% female, disease free at baseline) (5). This study found that median hs-cTnI concentrations changed from 2.6 ng/L to 3.4 ng/L over 10 years. Furthermore, the change in hs-cTnI values throughout 10-year follow-up more accurately predicted the cardiovascular risk in the general population than the most recent measurement (5). Indeed, considering the results of the most recent clinical studies, it is conceivable that an increase in hs-cTnI concentrations, even of only 5-10 ng/L over some months in a patient with a suspect of cardiomyopathy, should suggest an initial myocardial remodelling, ultimately culminating in symptomatic heart failure (3,4). Of course, an early and effective treatment of individuals at higher cardiovascular risk may revert the initial myocardial remodeling and slow down heart failure progression (3, 4). n conclusions, the results of some recent clinical studies have demonstrated that hs-cTnI and hs-cTnT methods are able to identify individuals at highest risk to develop symptomatic heart failure (3,4). However, further studies are needed to specifically evaluate the cost-benefit of screening programs specifically designed with the aim to identify asymptomatic individuals of the general population at higher risk for progression toward heart failure.

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SS04–02

VALUTAZIONE DEL RISCHIO CARDIOVASCOLARE IN PAZIENTI IN TRATTAMENTO CON CHEMIOTERAPICI

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Pazienti trattati con chemioterapia per patologia oncologica sono a maggior rischio di sviluppo di patologie cardiovascolari. Tali patologie sono rappresentate da aritmie, disfunzioni ventricolari, scompenso cardiaco, trombosi arteriosa, ischemia miocardica. La valutazione cardiologica del paziente, prima della somministrazione della terapia anti-neoplastica, rappresenta un'opportunità unica per l'inquadramento del paziente, permettendo sia l'evidenziazione di patologie pre-esistenti, sia l'implementazione di strategie atte alla prevenzione delle complicazioni correlate alla chemioterapia, sia durante che dopo la somministrazione.

Nella valutazione basale del paziente la determinazione dei marcatori cardiaci, troponina e peptidi natriuretici, fornisce dati oggettivi per una stratificazione del rischio di sviluppo di complicanze cardiache durante e dopo la somministrazione, identificando pazienti che possono trarre beneficio da trattamenti preventivi anche durante il trattamento chemioterapico. Incrementi dei marcatori non vanno interpretati come fattori che inducano ad una sospensione/riduzione delle dosi di chemioterapico, ma devono indurre una rivalutazione congiunta tra oncologi e cardiologi del paziente per indirizzarlo ad approfondimenti strumentali o a terapie preventive. Esistono infatti oramai parecchie evidenze che la somministrazione di ACE inibitori o di beta-bloccanti determina una diminuzione dei valori dei marcatori e soprattutto una significativa riduzione del rischio di sviluppo di complicanze.

Un punto ancora oggetto di dibattito è rappresentato dal timing dei prelievi. Mentre la determinazione basale è da tutti accettata, il timing e la frequenza di valutazione durante la chemioterapia non è ancora stabilita per nessun marcatore. Una strategia spesso percorsa è quella di una determinazione in associazione alle altre valutazioni ematochimiche, in maniera da poter monitorare in maniera seriale eventuali variazioni di concentrazione.

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SS04-CO09

PREVENZIONE PRIMARIA DEGLI EVENTI CARDIACI NEI DONATORI DI SANGUE: RISULTATI DI UNA ESPERIENZA MONOCENTRICA

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Premessa: Le malattie cardiovascolari sono la prima causa di mortalità (10,4% di tutte le morti) e la prima causa di ricovero ospedaliero (14.5 % di tutti i ricoveri), con un trend destinato a crescere secondo le ultime stime OMS. Realizzare in modo concreto un'azione educativa e di prevenzione di queste patologie puo# essere fondamentale per prevenire eventi cardiovascolari, soprattutto in quei soggetti con rischio medio-alto. L'utilizzo di test di laboratorio ad alta sensibilita# e specificità d'organo, come lo sono i metodi di ultima generazione per la misura delle troponine cardiache, permettono un'accurata valutazione del rischio anche nei pazienti con impegno cardiaco asintomatico. La possibilità di poter rilevare anche piccole concentrazioni di troponin a I in una popolazione sana , ha consentito di creare delle tabelle per la stratificazione del rischio cardiovascolare, differenziate per genere, e con rispettive raccomandazioni per rischio basso, moderato ed elevato. Data l'importanza di considerare la stratificazione del rischio come un fondamentale improvement nella gestione della prevenzione cardiovascolare si è scelto di eseguire ai donatori che afferiscono alla ST AOOR Villa Sofia-Cervello di Palermo, il test della troponina I in aggiunta ai test di screening per la qualificazione biologica previsti per legge.
Metodi: sono stati testati 3450 Donatori (2429 uomini e 1021 donne). Il test è stato effettuato sulla stessa provetta di siero del campione prelevato per le indagini di qualificazione biologica. Il test della Troponina I ad alta sensibilità (hsTnI) è stato effettuato su piattaforma Alinity (Abbott) con metodica CMIA. Sono stati avviati a consulenza cardiologica tutti i donatoricon valore di Troponina medio-alto.
Risultati: N.89 donatori, di cui 79 uomini (età: 32-65 anni) e 10 donne (età: 55-60 anni), sono stati avviati a consulenza cardiologica. Il valore medio di Troponina riscontrato, era di 6,8 e 22,2 per gli uomini e di 6,6 e 23,8 per le donne, indicativirispettivamentedirischio moderatoedelevato.Tali donatori presentavano i seguenti fattori di rischio: obesità, familiarità per malattie cardiovascolari e diabete, ipertensione arteriosa. Tutti i soggetti avviati a consulenza cardiologica, sono stati sottoposti a visita specialistica ed elettrocardiogramma;
n. 34 soggetti, con valori di Troponina elevata e con più fattori di rischio cardiovascolare presenti, sono stati sottoposti a successive indagini strumentali, quali test ergometrico, ecodoppler cardiaco. N. 2 soggetti sono stati ulteriormente sottoposti a RM cuore. In tali soggetti sono state individuate lievi patologie a carico del sistema cardiovascolare, prima di allora sconosciute, e sono stati tenuti in follow up per 6 mesi. Per tutti gli altri donatori avviati a consulenza cardiologica, non sono stati riscontrati dati clinici rilevanti e si è provveduto a dare indicazioni su uno stile di vita sano
Conclusioni: l'introduzione del test hsTnI, ha permesso di identificare precocemente i soggetti a rischio cardiovascolare all'interno della nostra popolazione di donatori di sangue attivando così un percorso di prevenzione primaria degli eventi cardiaci

SS05 Medicina personalizzata in ematologia, nuove frontiere

SS05-S01

PERSONALIZED MEDICINE IN HEMATOLOGY

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Hematology has paved the way for therapeutic innovations in medicine. This is also happening now in the new frontier of personalized medicine.
The definition and scope of the term "personalized medicine" varies widely, ranging from the extremely broad to the very narrow. Here we report several different definitions.
"The use of new methods of molecular analysis to better manage a patient's disease or predisposition to disease" by the Personalized Medicine Coalition; "Providing the right treatment to the right patient, at the right dose at the right time" by the European Union; "The tailoring of medical treatment to the individual characteristics of each patient" by President's Council of Advisors on Science and Technology; "Health care that is informed by each person's unique clinical, genetic, and environmental information" by the American Medical Association; "A form of medicine that uses information about a person's genes, proteins, and environment to prevent, diagnose, and treat disease" by the National Cancer Institute (NIH); "The use of genomic, epigenomic, exposure and other data to define individual patterns of disease, potentially leading to better individual treatment" by National Academy of Sciences (NAS).
Overall "precision medicine" is perhaps most synonymous to "personalized medicine".
But if we look closely at the evolution of hematology, the era of personalized medicine began several years ago.

- 1907: Reuben Ottenberg reports the first known blood compatibility test for transfusion using blood typing techniques and cross-matching between donors and patients to prevent hemolytic transfusion reactions.
- 1956: The genetic basis for the selective toxicity of fava beans ("favism") and the antimalarial drug primaquine is discovered to be a deficiency in the metabolic enzyme, glucose-6-phosphate dehydrogenase (G6PD).

- 1977: Cytochrome P450 2D6, a polymorphic metabolizing enzyme, is identified as the culprit for causing some patients to experience an “overdose” or exaggeration of the duration and intensity of the effects of debrisoquine, a drug used for treating hypertension.

Continuing through the years to the present day we can see many examples of personalized therapy that are current practice today: hemopoietic stem cell transplantation, infectious diseases prophylaxis and treatment, GvHD biomarkers, targeted drug levels, pre-emptive therapy on specific molecular targets, integration of targeted therapy inside transplantation program both as bridging therapy and early maintenance post-transplant particularly in acute myeloid leukemia because of the several mutation recognized determining clonal heterogeneity and drug resistance.

Real-world applications of immunotherapy are personalized medicine: allogeneic transplantation a platform for immunotherapy, checkpoint blockade treatments to pick-up anti-leukemia specific responses, CAR-T and TCR engineering, antibodies-redirected anti-leukemia specific responses, suicide-gene engineered T-cells and others.

Gene therapy, gene editing and CAR-T therapy are indubitably additional form of personalized therapy in which patients own cell, appropriately modified, are the therapeutic agent.

As final remark we must recognized limitations of personalized medicine. Several can be listed, in this abstract we wish to underline cost issue. Personalized medicine contributed to the recent rapid and impressive cost increment seriously impacting equity of resources distribution and therapeutic perspectives. As Physicians we should remember what may undoubtedly be the oldest but actual and most relevant definition of personalized medicine: it’s far more important to know what person the disease has than what disease the person has (Hippocrates).

SS05-CO10

SINGLE-MOLECULE REAL-TIME SEQUENCING OF THE M PROTEIN: TOWARD PERSONALIZED MEDICINE IN MONOCLONAL GAMMOPATHIES

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In patients affected by monoclonal gammopathies, tumoral B cells or plasma cells secrete a monoclonal antibody (M protein), which can be used to track the presence of the tumor itself. Moreover, the M protein can directly cause potentially life-threatening organ damage, which is dictated by the specific unique clonal light and/or heavy chain of each patient, as in patients affected by immunoglobulin light chain (AL) amyloidosis.

Patients’ specific M protein sequences remain mostly undefined and molecular mechanisms underlying M-protein related clinical manifestations are largely obscure. By combining the unbiased amplification of expressed immunoglobulin (Ig) genes through inverse PCR from cDNA with long-read DNA sequencing and bioinformatics analyses, we have established a method to unambiguously identify the full-length variable sequence of expressed Ig genes in patients with monoclonal gammopathies and to rank the obtained sequences based on their relative abundance, thus enabling the identification of the full-length variable sequence of M protein genes from a high number of patients analysed in parallel.

The assay, termed Single-Molecule Real-Time Sequencing of the M protein (SMaRT M-Seq), has undergone an extensive technical validation, including comparison with gold-standard techniques of immunoglobulin gene sequencing, assessment of reproducibility and sensitivity, and validation through proteomics on amyloid deposits. Noteworthy, SMaRT M-Seq successfully identified the full-length variable sequence of M protein genes from a cohort of 86 AL patients, including cases with a small clonal burden and an undetectable M protein with conventional diagnostic assays, validating its throughput. Sequence information was then exploited to enable the sensitive detection of clonotypic sequences.

High-throughput sequencing disease-associated M proteins from large cohorts of patients has the potential for uncovering molecular mechanisms of M protein-related clinical manifestations which, so far, have remained largely unexplored, and could enable approaches of personalized medicine for early diagnosis using sequence-based predictive algorithms and for detection of minimal residual disease.

SS05-CO11

EVALUATION OF THE CBC-O TOOL: WHY DOES THE MCHC INCREASE?

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Introduction: the increase of MCHC (Mean Corpuscular Hemoglobin Concentration) in CBC (complete blood count) is a complex problem, which must take into account numerous factors. RBC impedance and HGB optical density (photometric) measurements can be influenced by various causes, which can impact on red blood cell indices and may depend on both biological variables, such as: cold agglutinins, lipemia, jaundice, abnormal proteins, hemolysis, red blood cell disease, dehydrated red blood cells; that from pre-analytical factors such as delayed sample processing, temperature variations during collection, transport and storage. This paper evaluates the CBC-O (optical) tool, which supports the clinical pathologists in identifying the cause and in the correct reporting of the CBC parameters, avoiding the default thermostating of the samples.
Materials and methods: for the evaluation of the CBC-O tool, the samples, collected with K2EDTA anticoagulant, of 121 patients for the execution of the CBC were used. The tool can be calculated after performing the RET (reticulocytes) reflex analysis of the XN hematology analyzer (Sysmex) when MCHC is> 365 g/L. The tool CBC-O uses the parameters: RBC-I, RBC-O, HGB, HGB-O to calculate delta RBC, delta HBG and the RBC score through which the tool suggests the cause of the MCHC increase by returning the blood cell indices corrected reds of the CBC.
Results: the samples that have been used can be divided into different groups according to the cause of the MCHC increase: patients with probable presence of cold agglutinins, whose RBC indices obtained after thermostating the sample and those calculated by the CBC-O tool are been compared with overlapping results; with hemolysis / lipemia and samples whose MCHC was increased due to a delay in sample processing and problems related to transport and storage. In the latter, the comparison between the results obtained from the thermostating of the sample and the tool are not comparable.
Conclusion: identifying the cause of the increased MCHC allows the clinical pathologist to make corrections or make appropriate decisions.

SS06 - Sostanze psicoattive: dall'uso clinico all'abuso

SS06 - 01

NUOVI TREND DI CONSUMO DI SOSTANZE D'ABUSO DURANTE LA PANDEMIA DA COVID 19

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Per contenere e contrastare il COVID-19 (COronaVIrus Disease 19), le persone sono state costrette a rispettare misure severe come il lockdown e il distanziamento sociale, modificando i propri stili di vita. Questo evento ha avuto conseguenze negative sul benessere fisico e mentale delle persone, provocando stati di ansia e di rabbia, disturbi del sonno, depressione e disturbi da stress post-traumatico. Ciò ha portato sempre più individui a ricorrere all'utilizzo di sostanze sia lecite che illecite, con un aumento del consumo di alcol e di sostanze psicotrope, aggravando le condizioni di chi, già, soffre di tossicodipendenza (1). Anche, il mercato delle sostanze stupefacenti si è dovuto adattare alle restrizioni connesse al COVID-19, specialmente dopo la chiusura delle frontiere e le restrizioni nei viaggi. L'utilizzo di corrieri umani è stato, pressoché, sostituito da contrabbando tramite container intermodali o tramite catene di approvvigionamento commerciali (2). Perfino le rotte commerciali hanno subito modifiche, abbandonando il transito via terra a favore del trasporto via mare, come nel caso del traffico della resina di Cannabis prodotta in Marocco e trasportata nell'UE, prima della pandemia, via terra attraverso la Spagna. Al contrario, dalla seconda metà del 2020, sono stati segnalati ingenti sequestri di resina di Cannabis in numerosi porti marittimi europei. La pandemia, ha inoltre, accelerato la digitalizzazione del mercato delle sostanze stupefacenti, sostituendo la vendita per strada con metodi alternativi che hanno portato i consumatori a rivolgersi al mercato illegale del dark web o all'utilizzo di servizi di messaggistica criptati o a piattaforme web come Telegram o infine a servizi di posta con consegna a domicilio. Anche il tipo di sostanze stupefacenti consumate si è modificato. Mentre, l'analisi delle acque reflue, condotta nel 2019 nelle principali città europee, aveva indicato un aumento complessivo di consumi di sostanze come cocaina, MDMA, amfetamina e metamfetamina, droghe solitamente associate a eventi sociali ricreativi, durante la pandemia, l'interesse del consumatore si è spostato su ansiolitici e narcotici da consumare in solitudine. Oltre ai tranquillanti, è stato, segnalato un crescente utilizzo di oppioidi e di nuovi oppioidi sintetici. In Canada e in Nord America, dall'inizio della pandemia, si è registrato un aumento netto dei decessi per overdose da oppioidi sintetici come il fentanil (3) (in Canada, tra aprile e giugno 2020 sono stati superiori del 58% rispetto allo stesso trimestre del 2019). In Europa, si sono registrati cambiamenti nei livelli di abuso dei farmaci per il trattamento sostitutivo degli

oppioidi dopo la prima chiusura (4) nonché un aumento dell'utilizzo di droghe psichedeliche e dissociative, come LSD, 1P-LSD, 2C-B, NBOMes, ketamina, DMT e GHB. L'aumento di utilizzo non terapeutico di benzodiazepine e farmaci Z ha, anche, sollevato particolari preoccupazioni, sia per il basso costo sia per l'elevata disponibilità nonché per l'aumento di utilizzo tra i consumatori di stupefacenti ad alto rischio non consapevoli del contenuto e della potenza delle compresse acquistate online.

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SS06-02

RECREATIONAL AND CLINICAL USE OF GHB

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γ-hydroxybutyrate (GHB) is an illicit recreational drug of abuse acting as a potent central nervous system depressant and is often encountered during forensic investigations both in living and post-mortem cases. The sodium salt of GHB is registered as a therapeutic agent (Xyrem®), approved in several countries for the treatment of narcolepsy-associated cataplexy and (Alcover®) is an adjuvant medication for detoxification and withdrawal in alcoholics. Small amounts of GHB are produced endogenously (0.5-1.0 mg/L) in various tissues, including the brain, where it functions as both a precursor and a metabolite of the major inhibitory neurotransmitter γ-aminobutyric acid (GABA). Available information indicates that GHB serves as a neurotransmitter or neuromodulator in the GABAergic system, especially via binding to the GABA-B receptor subtype. Although GHB is listed as a controlled substance in many countries abuse still continues, owing to the availability of precursor drugs, γ-butyrolactone (GBL) and 1,4-butanediol (BD), which are often not under control. After ingestion both GBL and BD are rapidly converted into GHB (t½ ~1 min). The Cmax occurs after 20-40 min and GHB is then eliminated from plasma with a half-life of 30-50 min. Only about 1-5% of the dose of GHB is recoverable in urine and the window of detection is relatively short (3-10 h). This calls for expeditious sampling when evidence of drug use and/or abuse is required in forensic casework. The recreational dose of GHB is not easy to estimate and a concentration in plasma of ~100 mg/L produces euphoria and disinhibition, whereas 500 mg/L might cause death from cardiorespiratory depression. Effective antidotes to reverse the sedative and intoxicating effects of GHB do not exist. The poisoned patients require supportive care, vital signs should be monitored and the airways kept clear in case of emesis. After prolonged regular use of GHB tolerance and dependence develop and abrupt cessation of drug use leads to unpleasant withdrawal symptoms. There is no evidence-based protocol available to deal with GHB withdrawal, apart from administering benzodiazepines

SS06-03

NEW SYNTHETIC OPIOIDS

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In the last decades, several new psychotropic molecules mimicking the pharmacological effect of the classic drugs of abuse appeared on the illegal market, causing acute and fatal intoxications in more than 100 countries worldwide (1). These molecules are defined as new psychoactive substances (NPSs) by the United Nations Office of Drugs and Crime (UNODC) and their legal status is often controversial, although they pose an increasing public health threat (1) Recently, the subclass of new synthetic opioids (NSOs), in particular fentanyl and benzoimidazole analogues, stood out as an emerging class among NPSs and raised concerns due to the rapid increase of fatalities related to new analogues (1,2).
Similar to the classical opioids, namely morphine and heroin, NSOs selectively bind to the μ-, δ-, and κ-opioid receptors in the peripheral and central nervous system (CNS), thereby simulating the effects of endogenous opiates. These neurochemical interactions are mainly responsible of a classic opioid toxidrome characterized by miosis, sedation, coma, and even death, which can be reversed with the competitive antagonist naloxone (3)
In the early 2010s, USA were struck by the "opioid crisis" as NSOs flooded the illegal drug market and the number of opioid-related overdose deaths significantly increased in the USA, mainly involving fentanyl and analogues (3). In 2019, new NSOs subclasses (eg. benzimidazole opioids such as nitazene analogues), thiambutenes, and cinnamylpiperazines) emerged onto the illicit drug market, subsequent to the scheduling of fentanyl analogues in the USA and China (1).
In 2020, NSOs resulted the third largest group of NPSs identified by European Monitoring Centre for Drugs and Drug Addiction after stimulants and cannabinoid receptor agonists (1)

Similar situation was reported in Italy by the National Early warning system, in charge of
early detecingt emerging NPS threats in Italy, acquiring information from police forces and collaborative centrist raise an international alert (4).
From 2016 to 2019, 4 fentanyl analogs and 1 synthetic opioid (U47-700) were identified for the first time in Italy, following fatal intoxications. Among the identified fentanyl analogs, it is worth mentioning 4-furanyl fentanyl, identified for the first time not only in Italy but also in Europe.
The expansion of these NSOs constitutes an important challenge in clinical and forensic toxicology. First of all, most of these substances are not detected in laboratory routine screening tests and confirmation methods. Also, due to the low doses employed of these highly potent drugs, the concentrations expected in the biological samples are in the concentration ranges of low ng to pg/mL or ng to pg/g range, requiring extremely sensitive methods of detection (5).
The rapid identification of NPS is instead essential to stop the health and social problems related to the spread of these new dangerous and highly addictive substances in the general population and in conscious and unconscious consumers.

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SS06-CO12

THERAPEUTIC DRUG MONITORING OF MEDICAL CANNABIS IN PEDIATRICS: THE EXPERIENCE OF GIANNINA GASLINI INSTITUTE.

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The growing interest in medical cannabis-based therapies has increased in recent years and treatment of drug-resistant epilepsy is one of the most relevant applications. Medical cannabis is composed by the dried female flowering tops containing among others at least 90 cannabinoids. D9-tetrahydrocannabinol (THC) is the only psychotropic compound while cannabidiol (CBD) is the main non-psychotropic constituent. In recent years, a plant-derived pharmaceutical formulation of purified CBD oral solution (Epidiolex®) was approved as adjunctive therapy in conjunction with clobazam for drug resistant seizures associated with Dravet or Lennox-Gastaut Syndrome. The PK of CBD shows extensive variability posing the bases for treatment optimization TDM . Starting from 2007, we administered different available CBD preparations (eg. Italian FM2, Epidiolex®) and started performing TDM on dispensed products. We have developed several analytical methods for the measurement of THC and CBD and their main metabolites: 11-hydroxy-D9-tetrahydrocannabinol (11-OH-THC), 11-nor-9-carboxy-D9-tetrahydrocannabinol (THC-COOH) for THC and 7-hydroxy-cannabidiol (7-OH-CBD), cannabidiol-7-oic acid (7-COOH-CBD), 6-alpha-hydroxy-cannabidiol (6-a-OH-CBD), and 6-beta- hydroxycannabidiol (6-b-OH-CBD),for CBD. , in plasma as well in microsampling volumetric devices (VAMS) using ultra performance liquid chromatography coupled
to tandem mass spectrometry (UHPLC-MS/MS) and validated them following international guidelines. Several clinical samples derived from pediatric patients under treatment with both medical cannabis and CBD-based pharmaceuticals were analyzed and differences in blood levels of THC and CBD and their respective metabolites as well as correlation with administered dosages were studied. VAMS have been also validated in capillary blood posing the bases for further developing the possibility of home sampling and minimizing the discomfort caused

by venipuncture. Results put in evidence the high inter-individual variability and large differences in bioavailability between purified and galenic preparations underlying the need to implement TDM for personalization of medical cannabis treatments.

SS06-CO13

HS-SPME-GC-MS ASSESSMENT OF THE EXPOSITION TO THC DURING SMOKING BY A PORTABLE VAPORIZER FOR CANNABIS

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The emerging hemp industry is interested by the new tabletop or portable vaporizers as they appear less harmful of the mainstream cigarettes. They work heating dried materials at low temperature in order to achieve an aerosol full in active compounds like phytocannabinoids, flavonoids, and terpenoids without combustion by-products. Investigate the performance of a new pocket pen vaporizer was the aim of this study. In order to evaluate the potential exposure of vapers, a HS-SPME- GC-MS method was developed for the characterization of vapor phase produced by cannavaping. Vaping was realized under standard conditions and vapor phase was collected in quick and direct manner to avoid any degradation and contamination. Analytes sampling were carried out by a DVB/CAR/PDMS fiber, and their identification was performed through gas chromatography coupled to mass spectrometry. This approach allowed to monitor active ingredients and to verify absence of harmful compounds which characterize the mainstream cigarette smoke. In addition, the quantification of the main cannabinoids, THC and CBD, in the vapor phase was performed in order to evaluate their potential inhalation by vapers. Results confirm the ability of this new portable vaporizer to release cannabinoids and terpenes in a vapor that is free from the by-products of combustion making it a valuable tool for cannabinoids administration in medicine.

SS07 I POCT: uno degli strumenti "per una nuova normalità"

SS07-01

ESPERIENZA DI IMPLEMENTAZIONE E GESTIONE DEI POCT IN UNA ASL ABRUZZESE CON 5 PRESIDI

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Scopo del lavoro è stato quello di creare una rete territoriale di POCT nei presidi di pronto soccorso degli Ospedali periferici dislocati nel territorio interno abruzzese. Come preparazione del lavoro è stata completata una ricognizione su tutti i presidi e successivamente sono stati installate 6 strumentazioni di POCT nelle sedi di Atessa, Casoli, Guardiagrele, Gissi ed Ortona.Su ciascun presidio si è provveduto ad installare uno strumento per l 'esame emocromocitometrico, uno per gli esami di base di chimica-clinica, uno per l' esame delle urine, uno per i test di base della coagulazione, uno per la troponina ed un emogasanalizzatore.Si sono poi definite le procedure per l 'esecuzione dei controlli sulle varie strumentazioni. Inoltre è stata definita una procedura aziendale per la Gestione dei POCT.Tutto il progetto, per il suo avvio, ha richiesto 6 mesi da settembre 2021 a marzo 2022. La rete territoriale installata sui vari presidi viene gestita dall'ospedale di riferimento,ossia quello dell 'Ospedale Clinicizzato SS.Annunziata -Chieti dove personale dedicato si occupa della verifica di tutte le strumentazioni.

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SS07 - 02

SOLUTIONS AND PATHWAYS IN A LEVEL III PEDIATRIC HOSPITAL

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In compliance with ISO standards 15189 and 22870, the governance of decentralised diagnostic (POCT) is one of the new challenges for many laboratories in Europe. The path requires organisation and specific skills, and the aim is to ensure analytical systems that combine three fundamental aspects: ease of use, analytical quality, and process traceability. POCTs are needed in intensive care, including neonatal and pediatric emergency/urgency wards, ensuring immediate clinical picture framing and rapid assessment and prognosis, essential for patient outcome. The appointment of a POCT Manager for

A.O.U. Ospedali Riuniti Ancona allowed a reorganisation process to be undertaken, starting from the Pediatric Hospital G. Salesi. A step-by-step process was implemented that provided the establishment of a POCT group, training in the use of the system to support and assist the departments in managing the critical testing phase. Crucial elements of the project were: the careful selection of instruments, which tend towards an analytical "operator-independent" solution; the connection to the middleware, guaranteeing the management of the Acute Care systems present; the compulsory acceptance of tests on the order entry system, with bar-code printing for the sample/patient's identification; the preparation of an ad hoc report for the tests. For performance monitoring, indicators relating to percentage of accepted and correctly identified samples; adverse events due to pre-analytical errors; effectiveness of training of department operators, through specific Key Performance Indicator (KPIs) were identified. The intention of the new organizational model is to move from a vertical logic of care, that is summative of individual performances and services, to a horizontal patient-oriented, socio-assistance logic, using the most suitable technological and IT solutions. Diagnostic and IT technological supervision, by the Analysis Laboratory, allow complete control of the decentralised location, with guaranteed analytical quality and traceability of the services provided. The new trends taking place in the health context, demographic change, increased user expectations of good health, development of new health technologies, produce a greater demand for health services with an increase in treatment and care costs. This diagnostic uncertainty, if not well addressed, could lead to inappropriate prescriptions, unnecessary additional tests, and to an increase in potentially avoidable hospital admissions. POCTs can improve the diagnostic process and medical prescriptions, increase the quality of care and, indirectly, relieve pressure on healthcare systems, by shifting the axis of care from the hospital to the territory ('comfort zone'). At the same time, for chronic pathological conditions (frail patients), these tests provide an immediate result with early adaptation of therapy. For children, clinical decision-making process and its complexity are different from those for adults. The impact of POCTs on patient outcomes and healthcare processes in pediatric care will therefore need further investigated.

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SS07-03

POINT OF CARE TESTING, CONNECTIVITY AND REGULATIONS: ALL TO BE IMPLEMENTED AT THE SAME TIME AS A "KEY SOLUTION"

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In a changing world Health reform (especially primary care reform) is now a focal point of attention in many countries, for a number of reasons. Access to care is frequently limited due to disability, distance, service is fragmented and disconnected, error rates are unacceptable, evidence and adherence to guidelines are poor and patient experience is irrelevant. Managers and policymakers should identify inefficiency and ineffectiveness in primary care, services should be redesigned through the use of a care pathway-based approach, physicians and other caregivers should be in position to make decisions and take action at the first point of contact with the patient at local clinics, nursing homes and primary care facilities. POCT can play an important role in "problem solving" policy, because they offer numerous advantages. However, using those devices in settings outside of the hospital pose critical and important clinical risks. The most important aspect for POCT is how to use them, to address many of the problems that arise from disjointed services and delays in delivering vital information, such as medical test results, everyplace those results are needed. Delocalization does not imply absence of control, which is always mandatory, but without connectivity it is impossible to obtain an effective management. The integration of POCT data into the patient's electronic medical record is a critical component of management. Connectivity of POC devices supports documentation of results, continuity of care, and enables data review for quality assurance compliance. The Clinical and Laboratory Standards Institute (CLSI) produced guidelines on the requirements that should be met when manufacturers are developing the connectivity features of the device. Incongruously, even the ISO 15189 and 22870 do not recognize connectivity as a mandatory requirement for accreditation, despite the strong commitment to traceability and quality control. In Italy a national rule is lacking and local regional rules are rather divergent: some of them contain only essential indications while others are too detailed and difficult to apply, moreover most of them are timeworn. Only recently (2021) the Regione Campania emanated a regional decree. The lack of harmonization and regulation among Italian regions makes it necessary to issue of a national rule aimed at minimizing the problems associated with POCT management. Moreover, if not taking part in development and management of POCT Systems, a big opportunity is being lost for laboratorians to increase their professional role, experience, knowledge and scientific contribution to the POCT domain.(CLSI POCT01-A2 Point-of-Care Connectivity, 2006; CLSI AUTO09-A: Remote Access to Clinical Laboratory Diagnostic

Devices via the Internet; Approved Standard, 2006 CLSI AUTO11-A2: Information Technology Security of In Vitro Diagnostic Instruments and Software Systems; Approved Standard – Second Edition, 2014).

SS07-CO14

IL POCT NELLO SVILUPPO DI UN NETWORK CLINICO ASSISTENZIALE NELLA MEDICINA DI PROSSIMITÀ

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Point of care (POC) are tests conducted near the site of patient care, outside of laboratory, usually performed by patients or healthcare personnel not trained in laboratory medicine. POC testing (POCT) require small sample volumes, minimize pre-analytical errors, and reduce alterations of labile analytes. Furthermore, when used appropriately, could improve the patient's outcomes providing faster results and earlier therapeutic strategies. Instead, its over or incorrect use could lead to a patient risk and potential increase of healthcare costs.In the Province of Modena, a decentralized and multi- professional POCT diagnostic system was developed to integrate the clinical and diagnostic pathways supporting fast clinical decisions, to improve outcomes and to optimize the clinical patient pathways. We installed 129 POCT (77 emogasanalyzers, 9 coagulometers, 9 instruments for haematological tests, 9 instruments for chemical analyses, 10 counters, 6 for immunoassay, 3 cube-PCR and 6 for rapid diagnosis of COVID-19). These instruments were installed not only in emergency rooms of three Hospitals but also in specific setting as the OSCO-Community hospital, oncological and emergency setting and Neonatal Intensive Care. Furthermore, 264 glucometers were located in various departments of three hospitals. This system provides multiple advantages for: (i) patients reducing waiting time and long-distance travelling from home, (ii) healthcare professionals improving skills, autonomy and responsibility, reducing workload and promoting collaboration, and (iii) the healthcare system giving continuity in the therapeutic and assistance pathways ensuring a high analytical quality and costs reduction. To implement a successful POCT network is vital to develop a multidisciplinary team composed by physicians, nurses and laboratory technicians working together. Furthermore, the continuous training and education of operators guarantees the efficiency and productivity of the system.In conclusion, the POCT network represent an opportunity for the health care system to consolidate the patients-based laboratory medicine, reducing for diagnostic response, clinical decision and the hospitalization.

SS07-CO15

ANALYTICAL PERFORMANCE EVALUATION OF A POINT OF CARE TESTING SYSTEM FOR THE COMPLETE BLOOD COUNT

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Introduction. Point of care testing (POCT) is an alternative approach to laboratory testing, that should be preferred only when the test TAT must be so short that samples management in laboratory is not suitable. However, POCT should generate results comparable to those obtained in laboratory. Purpose of the study. In our study we estimated the concordance of the blood cell count carried out on POCT analyzer Norma Icon-5 with that performed in laboratory on automated blood cell count Sysmex XN1000, to evaluate the POCT implementation in Emergency Department (ED). Methods. Venous blood samples were collected in tubes containing K3EDTA anticoagulant to 40 patients admitted to the ED. All samples were assayed on Norma Icon-5 and, within 10 minutes, on Sysmex XN1000. The degree of concordance between POCT results of red blood cells (RBC), white blood cells (WBC), and platelets (PLT) count and haemoglobin (Hb) concentration was evaluated. Data were compared with Passing-Bablok regression and Bland-Altman plots. Results. The PoCT vs. XN1000 bias was -2,44% (95% IC: -3,45÷-1,43), 0,16% (95% IC:-0,70÷-1,92), 1,22% (95% IC: 0,612÷1,83) and -10,45 (95% CI -12,33÷8,67)% for RBC, WBC, Hb and PLT respectively. PoCT vs XN1000 absolute bias was -0,11 (95% IC -0,15÷-0,06), 0,07 (95% IC -0,01÷0,16), 1,65 (95% IC 0,80 ÷ 2,45) e -21,25 (95% IC -24,40÷-18,11) for RBC, WBC, Hb and PLT respectively. Passing- Bablok regression equation was y= -0,015 (-0,309÷0,240) + 0,976x (0,915÷1,0435), y= -0,186 (-0,404÷0,012) + 1,038x (1,008÷1,068), y = -1,889 (-7,224÷1,500) + 1,030x (1,000÷1,069) e y=-12,098 (-21,500÷-3,824) + 0,964x (0,924÷1,000) for RBC, WBC, Hb and PLT respectively. Conclusion. The POCT blood cell counter results were statistically concordant with those of the laboratory system only for WBC e RBC count, but showed a clinically acceptable bias also for Hb concentration values. However, PLT showed an underestimation of PLT statistically significant and also exceeding the minimum clinical allowable bias. Therefore, the POCT blood cell counter can be used for clinical purpose only for RBC, WBC and Hb determination.

SS08 - Diagnostica delle crioglobuline

SS08-01

CRYOPROTEINEMIA: THE NEW FRONTIER FOR THE LABORATORY

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Cryoglobulinemia is a rare pathologic condition that can be difficult to diagnose both clinically and, in the laboratory, which is why close collaboration between the clinic and laboratory is essential. The laboratory needs the skills and experience to interpret the laboratory tests and the clinician should not hesitate to contact the laboratory when the result is not supported by the clinical signs. To strengthen this collaboration, the Protein Study Group of the Italian Society of Clinical Biochemistry (SIBioC) in collaboration with the Italian Association for the Fight against Cryglobulinemia (ALCRI) have established a fruitful partnership.
The laboratory plays a central role in the cryoproteinemia diagnosis, but the laboratory criticalities are well known. In the pre-analytical phase, maintenance of the heat chain is a critical issue; from the withdrawal until the arrival in the laboratory the sample has to stay at a temperature of 37°C. In the analytical phase, the sample must be kept at 4°C to allow the cryoproteins to precipitate, then, the cryoprecipitate must be washed, dissolved and immunotyped. The post-analytical step is cryoprotein type identification which may be subject to the operator's interpretation.
Cryoprotein testing remains totally manual and operator-dependent so it was important to identify areas where best practice guidance or even harmonisation of the laboratory investigation would be beneficial.
Patients with untreated cryoproteins can suffer significant tissue damage leading to limb amputation or even death, so the importance of cryoprotein analysis cannot be underestimated. Cryoproteinemia is in fact a pathological condition whose diagnosis cannot be made without laboratory findings. However, it is undeniable that cryoprotein research suffers from numerous pitfalls involving all stages of the analytic process. These criticalities are exacerbated by the strong inter-laboratory heterogeneity often conditioned by local technical and organisational resources. It is, therefore, crucial to urge laboratories to participate in External Quality Assessment (EQA) programmes for cryoproteins, bearing in mind that the only one currently available is the one proposed by UK-NEQAS.
Still uncommon is the cryofibrinogen analysis, the determination of which would be complementary to that of cryoglobulins, not least because of the overlap in symptoms between cryoglobulinemia and cryofibrinogenemia. This test, however, which is not included in the Essential Levels of Assistance of Italian healthcare, is slightly or not at all requested by clinicians, and consequently, laboratories rarely equip themselves for this determination, thus deterring demand.
In summary, there is a need to start a process of harmonising analytical procedures for cryoproteins testing by a continuous dialogue between clinics and laboratories with the frontline commitment of scientific societies. In particular, the path taken by SIBioC and ALCRI is precisely that of collaboration in order to draw up recommendations useful to the laboratory technician, the clinician and above all the patient.

SS08-02

CRYOGLOBULINEMIA AND THERAPY

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Abstract: Treatment of patients with cryoglobulinemic vasculitis (CV) should be tailored to the single patient, considering the severity of the disease. Expertise, knowledge of biology, and multidisciplinary cooperation are needed (1). Furthermore, the approach is substantially different in HCV-related or -unrelated CV, as well in the course of CV with type II or III versus type I cryoglobulinemia (2). Comorbidities and the previous therapies for CV, in particular corticosteroids and cyclophosphamide, and for HCV infection must be considered. Moreover, the irreversible organ damage, ie., in the case of peripheral neuropathy or nephritis, caused by parenchymal fibrosis after vasculitis requires supportive therapy (1).
In life-threatening manifestations, early diagnosis and prompt treatment are mandatory (3). High-dose corticosteroids and plasmapheresis represent the most rapid approaches currently available (3). Rituximab and cyclophosphamide remain further options to spare corticosteroids. In very severe, HCV-related CV, antiviral therapy is not the first priority. The risk of infections during treatment is high. Prophylaxis and early recognition and treatment of infectious complications are mandatory. Motor neuropathy and rapidly progressive glomerulonephritis also deserve an aggressive approach. In severe manifestations of CV and in patients recovering from a life-threatening CV, rituximab represents the best immunosuppressive treatment. Mycophenolate mofetil may be an alternative in some patients with milder renal disease. The efficacy and safety of rituximab in CV are supported by controlled, randomized trials (1) and worldwide 10-year real-life experience. The concomitant use of corticosteroids can be substantially reduced (1). Rituximab does not worsen HCV infection in the long term. By contrast, rituximab may induce severe reactivation of HBV infection (4). In about half of patients, the duration of the response to a single cycle of rituximab may be quite long (4). Regular maintenance schedules appear advisable, however, when a relapse would be hazardous. Cryoglobulins and rheumatoid factor (RF) decrease but usually do not disappear after rituximab treatment, however, controlling the burden of the B-cell

lymphoproliferation is usually sufficient (2). Antiviral therapy with Direct Antiviral Agents (DAAs) has the strongest biologic rationale in HCV-related CV. They may be added even ab initio to rituximab. Such a combination might show synergistic effects. In HCV-related CV, DAAs should be given whenever possible. Complete clinical response requires a sustained virologic response. Cryoglobulins and RF usually decrease over time and may even disappear, although CV may also persist, consistent with a true autoimmune disease (2).
Many patients with CV present non-severe manifestations, consisting of purpura, arthralgias, and weakness. Because the clinical picture may be highly variable based on disease chronicity, the frequency of relapses, the age of the patient, the disease activity, the concomitant liver disease, and comorbidities, the treatment must be individualized (5).

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SS09 - CASI CLINICI selezionati da Abstract

CC01

UNA COMPLICATA DEFINIZIONE DEL RISCHIO NEOPLASTICO LEGATO A BRCA

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Low-frequency constitutional gene variants represent alterations that inform inherited cancer risk definition and precision medicine strategies in cancer. Patients diagnosed with ovarian cancer should be first tested for mutations in BRCA1/BRCA2 genes on the neoplastic tissue to predict the response to platinum-based agents and PARP inhibitors.
In case of a positive result, the pathogenic mutation is searched for in the matched DNA isolated from peripheral blood. The constitutional origin and the zygosity of the mutation have to be ascertained to define hereditary cancer risk, implement surveillance and preventive strategies and assess cancer risk for family members. If a pathogenic genomic variant is present but with a low frequency in the DNA from peripheral blood, this may be ascribed to an event of clonal hematopoiesis of indeterminate potential or to a mosaicism. Analyses of secondary normal tissues are therefore necessary to exclude sequencing artifacts and confirm the presence of the low-frequency pathogenic variant in blood or even in other non-transformed tissues. The clinical case of an ovarian cancer patient with a triple negative breast cancer history was analyzed along with her family members.
The DNA from the patient's ovarian carcinoma was isolated and NGS-sequenced for BRCA1/BRCA2 genes. A BRCA1 nonsense pathogenic mutation was identified and its presence confirmed in the DNA from peripheral blood, though with lower-than-expected heterozygous frequency. Further NGS-based analyses in secondary normal tissues revealed the patient as a constitutional mosaic for this variant. In addition, both her breast and ovarian neoplastic tissues harbored this variant with high frequency, demonstrating that the mosaic variant contributes to tumor etiology. A screening of family members revealed that both parents were negative for the pathogenic mutation, confirming therefore the mosaic as a post-zygotic event. One of the daughters, finally, resulted as a previously undiagnosed heterozygous carrier for the pathogenic variant, indicating that the germline tissue of the mosaic patient was involved and the low-frequency BRCA1 variant was heritable.
Constitutional mosaicism is a renowned mechanism for multiple hereditary cancer-associated genes and enables access to personalized therapies and preventive cancer strategies. Therefore, to improve the current standard of constitutional analysis, we propose a new algorithm for the BRCA diagnostic routine in order to increase the sensitivity of germinal assessment and decrease the number of false negatives when pathogenic or likely pathogenic variants occur at low-frequencies.

CC02

UN CASO COMPLESSO DI ATTRIBUZIONE DI PATERNITÀ

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The analysis of STR loci is one of the most powerful methods to estimate relatedness between individuals. In the current case, we describe a complex paternity case arrived in Forensic Genetics Laboratory of Ceinge. A woman requested a paternity testing to know whether a man was the father of her child. Buccal swabs were used to obtain DNA specimens. Autosomal profiles of all subjects were obtained with GlobalFiler™ Amplification kit. Our results showed that child's autosomal profile presented a mixed profile with a maximum of three alleles per locus. Excluding possible sources of contamination, this scenario was compatible with a mother/son mixture. In fact, the child, because affected by leukaemia, previously had undergone to an allogeneic hematopoietic stem cell transplantation with his mother as donor. The PowerQuant® System used to quantify DNA samples, highlighted a concentration autosomal/Y ratio of almost 2. This finding was also underlined with the GlobalFiler™ system, which showed, in Amelogenin locus, a peak height ratio XX/XY of 1.8. We did not have the recipient's autosomal profile, prior the transplant, therefore, we compared locus by locus, the known maternal profile and the mixed child profile. We identified the pairs of mother-son alleles for each locus applying the deconvolution rules of two contributors in the mixture, where one of them is known. Hence, we chose the most probable pair, considering the profile mixture ratio woman/man of 1.8. According to International Society for Forensic Genetics guidelines (ISFG), the biostatistical interpretation was based on a likelihood ratio (LR) approach. Furthermore, LRmix software was used to interpret the mother/son mixture and Familias 3 to comprehend the kinship on the autosomes analysis. In the end, the father/son relationship was confirmed by Y-STRs analysis. Our case highlights that, although buccal swabs represent the most used tool to collect DNA in forensic investigations, we should bear in mind the possible occurrence of chimerism in reference material derived from these samples, if a subject had an allogeneic hematopoietic stem cell transplantation. Therefore, in similar case, other specimens, like hair roots, could be used if they are available.

CC03

IL RUOLO DEL LABORATORIO NELLA VALUTAZIONE DI UNA INASPETTATA ALTERAZIONE DEL APTT

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Acquired haemophilia A (EAA) is a rare hemorrhagic syndrome with autoimmune pathogenesis, due to the development of autoantibodies directed to various epitopes of the Factor VIII molecule. Early recognition of the pathology could help in the adeguate management of clinically suspected EAA, thus a specific laboratory diagnostic algorithm is needed in case of prolonged APTT.Clinical case: on February 2020, patient S.L., 80 years old female patient, accesses to emergency department due to lipothymia, and diffuse hematomas on legs and shoulder since about a week. She underwent standard hematologic tests which show Hb dosage of 7.1 g/dl and normal coagulation parameters except APTT ratio =1.6 . She was not under anticoagulant therapy. APTT ratio was ridetermined the following day, confirming a value 1.4. EAA is suspected thus mixing test and Factor VIII dosage were requested. The mixing test was performed after an incubation time of two hours at 37 ° of a 1:1 mixture of patient and normal plasma confirming a prolonged an APTT ratio of 1.9, thus without correction, and Factor VIII dosage of 5%. Obizur was administrated with excellent clinical follow up. However, the observed delay in performing an optimal diagnostic test could have compromised patient's prognosis. To overcome this output, a laboratory diagnostic algorithm has been proposed as a kind of reflex testing that can be automatically performed by the laboratory staff in case of extended APTT, when a suspicion is referred by clinicians based on clinical and anamnestic (i.e. no anticoagulant therapy) information. The use of APTT/ mixture test could represent a firstlevel approach that should be performed, even in an emergency mode. Failed or incomplete correction of the APTT after mixture test performance suggests the presence of an interfering antibody and orient the choice of appropriate therapy.Acquired haemophilia A is a rare autoimmune disease (1.5 cases / 106 inhabitants / year) secondary to the production of autoantibodies inhibiting factor VIII and is burdened by a mortality of up to 30%. Diagnosis must be timely. We propose a scheme of reflex testing immediately performed by the lab when the clinical and anamnestic information collected by the doctor in charge of the patient rise the suspect a coagulation problem of unjustified or not known cause.

CC04

UN POSSIBILE EFFETTO AVVERSO DELLA VACCINAZIONE ANTI SARS-COV-2

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Background: SARS CoV-2 vaccines, which demonstrated a high efficacy and a beneficial safety profile, could also represent a trigger factor for immune-mediated disease. We report a case of severe anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis following the mRNA vaccine for COVID-19, diagnosed de novo in February 2022 in a Nephrology Unit of Southern Italy.
Case Report: A 21-year-old man of Moroccan origin, with a history of bronchial asthma, entered the hospital for respiratory distress and low-grade fever, occurred few days after the first administration of the BNT162b2 vaccine. He was discharged with oral steroid and antibiotic therapy. One day after the second dose, he entered the hospital again due to worsening of respiratory symptoms, fever, edema and papulo-erythematous/purpuric lesions on limbs and trunk. He underwent routine blood tests with these findings: hypereosinophilia (6.63x10³cell/µL), increased creatinine (2.1 mg/dl), proteinuria (6 g/24h) and microhematuria (>1.0 mg/dL). Further laboratory tests showed increased levels of total IgE (6474 IU/mL, normal value <100 IU/mL) and Eosinophilic Cationic Protein (109 µg/L, normal value <13 µg/L). Autoantibodies anti Myeloperoxidase (Anti-MPO) were positive with high levels (740 CU/mL, cut-off value <20 CU/mL). Additional instrumental examinations and renal biopsy confirmed the diagnosis of ANCA-associated vasculitis. The patient was treated with metilprednisolone (3 boluses of 1g/day), then prednisone (1mg/kg/day) and 2 boluses of Rituximab (375mg/m²). After two months, the follow-up exams revealed normal serum creatinine level (0.8 mg/dl), reduction of proteinuria (3 g/24h), negative anti-MPO and complete remission of the respiratory status.
Conclusions: Among the complications of anti COVID-19 vaccines, several cases of de novo or relapsed ANCA-associated vasculitis were recently reported. We described a new case, onset in a younger manthan previous studies. Even though the relationship between COVID-19 vaccination and development of autoimmune disease has only been suggested, an appropriate laboratory approach, in support of clinical surveillance for immunological complications, should be helpful to diagnosis, monitoring and clarification of possible physiopathologic connection.

CC05

GAMMAPATIA MONOCLONALE E INFEZIONE DA SARS-COV-2

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We report the clinical case of a 90-years-old woman with a history of arterial hypertension, hypothyroidism, obesity, and chronic obstructive pulmonary disease (COPD), who referred to our center (Pederzoli hospital, Peschiera del Garda, Verona, Italy) for dyspnea in January 2022 after tested positive for SARS-CoV-2 infection. The patient received primary vaccination with mRNA vaccine in October 2021. At the time of hospital admission, chest high resolution computed tomography (HRCT) revealed bilateral ground-glass opacities compatible with interstitial pneumonia. Blood tests revealed lymphopenia (0,7 10^3/ uL), iron (33 ug/dL) C-reactive protein (CRP) (28.1 mg/dL) and erythrocyte sedimentation rate (ESR) (79 mm). Treatment with oxygen therapy, steroids, and immunosuppressants (Baricitinib) was started. Two weeks after hospital discharge, during a routine examination, a totally unexpected alteration of the electrophoretic pattern was found on serum protein electrophoresis (SPEP) performed with Capillarys 2 (Sebia, Lisses, France). This alteration is visible as apeak in the g region and it was characterized as IgG- kappa by immunofixation performed with Hydrasys 2 (Sebia, Lisses, France). In this case, the unforeseen occurrence of such alteration in SPEP caught our attention because the patient had no previous evidence of monoclonal gammopathy, and her SPEP was normal in January 2022.A few studies describe the occurrence of a monoclonal spike in association with acute inflammatory illnesses, especially in viral infections. Considering that during severe COVID-19 there is a release of IL-6, which plays an important role in B-cells differentiation into plasma cells, a correlation could be hypothesized between the COVID 19 disease and the alteration in SPEP, without excluding the possible interference given by pharmacological therapy.In conclusion, our findings underline the need of further studies in order to evaluate the degree of immune hyperactivation in patients with severe COVID-19 and the prognostic role to improve the management of patients in this clinical setting.

CC06

UN LIQUIDO SINOVIALE DA INDAGARE ATTENTAMENTE

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Paziente di 82 anni con gonalgia ingravescente, deambulazione difficoltosa e dolore alla flessione al ginocchio destro portatore di protesi totale. Il ginocchio è instabile agli stress in varo-valgo e alla radiografiasi presenta mobilizzazione franca di entrambe le componenti tibiali e femorali. Si pone indicazione alla revisione dell'impianto. Ves elevata, scollamento di entrambe le componenti e storia clinica di dolore rendono necessario escludere un'infezione periprotesica. Si esegue aspirato di liquido sinoviale per conta cellulareed esame colturale. Il liquido, di aspetto torbido e colore paglierino, è raccolto in provette K3EDTA trattato con ialuronidasi e analizzato con contaglobuli XN 2000 Dasit secondo le raccomandazioni CLSI e ICSH. La conta automatizzata mostra un numero elevato di cellule 85000 uL di cui 83% mononucleate e 17% polimorfonucleate senza allarmi morfologici o strumentali. I dati suggeriscono una infezione periprotesica. Lo scattergram strumentale è di colore grigio e forma allungata e mostra una popolazione unica di elementi cellulari la cui peculiarità allerta gli operatori di laboratorio. Si allestisce quindi camera di Burker e cytospin. Sia la conta in camera che il vetrino mostrano assenza totale di elementi figurati e presenza in grande quantità di materiale amorfo, di colore biancastro, somigliante a materiale plastico. La conta al contaglobuli sebbene validata e affidabile soffre di alcune interferenze. In questo caso frammenti plastici amorfi generano segnali captati dal contaglobuli come cellule fornendo un dato numerico errato. Questo evento va considerato se il tipo di impianto è soggetto a usura precoce o se instabilità e mobilizzazione generano alterata cinematica articolare con formazione di particolato anomalo. Basandosi solo sulla conta automatizzata il paziente sarebbe stato classificato come infetto esponendo il clinico ad una diagnosi errata ed il paziente ad una procedura chirurgica aggiuntiva non necessaria. L'approccio ragionato al campione è fondamentale per gestire il rischio di diagnosi errate. In questo caso l'approfondimento al microscopio ottico ha permesso di evidenziare la presenza dell'interferenza ed evitare un danno al paziente.

CC07

I REPERTI INSOLITI NECESSITANO DI INDAGINI APPROFONDITE

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Factitious disorder imposed on another (FDIA), also known as Munchausen syndrome by proxy, is a relatively rare, underreported, but serious form of child abuse. Deception is the core of this behavioral pattern. It is characterized by the falsification of signs/symptoms or induction of injury or disease by a caregiver (generally the mother) without external reward (1).Here, we report a case of FDIA in which laboratory findings were crucial for a timely diagnosis. A 1.5-year-old male child was hospitalized multiple times, mainly because of "intermittent gross hematuria". The urine samples, collected at home and brought in by the mother, appeared red, but, in some occasions, also pink, gray and violet. In two cases the mother reported the presence of blood in the stools and fever. Urinary tract echography, laboratory tests for renal function and metabolic diseases were unrevealing. When once again accessing the emergency room, the mother provided two urine samples with a highly concentrated sediment of red color and a diaper with fine sand-like 'brick dust'. After centrifugation the supernatant was clear yellow and free hemoglobin and myoglobin were absent. Microscopic sediment examination showed viscous large red spots suggesting the presence of an exogenous contaminant. Urine infrared spectroscopy (IRS) revealed traces of seed, crumb and bread crust, and chromatographic analysis showed high levels of lycopene, a major component of tomato sauce and tomato-based food products. A diagnosis of FDIA was done and actions to protect the child's health were taken. Despite the presence of highly suggestive warning signs or red flags in both the caregiver and the victim, FDIA diagnosis is generally difficult and often complicated by a real underlying disease, and can take several years of observation. The reported mortality rate related to FDIA ranges between 6 and 9%. Therefore, early diagnosis is of great importance in order to limit morbidity and mortality. Major efforts to identify exogenous contaminants of biological fluids, if suspected, should be undertaken.

1) Bass C, Glaser D. Lancet. 2014 Apr19;383(9926):1412-21.

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LA SPETTROSCOPIA PROTONICA DI RISONANZA MAGNETICA NUCLEARE COME BIOPSIA BIOCHIMICA VIRTUALE

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Introduction

Myotonic dystrophy type 1 (DM1) is a multisystem disease caused by a (CTG)n expansion in the gene coding for DM protein kinase (DMPK) on chromosome 19q13.3 [1]. Diffuse morphological and functional brain white matter (WM) changes [2], mainly in frontal, temporal and parietal lobes, are reported in neuroimaging and pathological studies [3].
In vivo proton MR spectroscopy (1H-MRS) is a quantitative technique that allows to assess non- invasively the metabolism of targeted brain volumes of interest [4] (VOIs).
Our case pointed to the diagnostic role of 1H-MRS as "virtual biochemical biopsy" for the characterization of WM changes in a DM1 patient.
Methods: The patient (M/36 years) presented a juvenile form of DM1 (genetic class E2) with a disease duration of 24 years. As part of disease monitoring that included clinical and neuropsychological evaluations, he performed a brain morphological MRI that showed a suspected low grade glioma in the right parieto-occipital WM.
Using a standardized protocol on a high field 3T scanner, 1H-MRS was localized in four VOIs [5] (8ml, each scan duration ~3min): 1. suspected glioma, 2. likely DM1 related occipital WM alteration, 3. and 4. contralateral to 1. and 2. morphological healthy tissue. Metabolites content was quantified with the automatic software LCModel 6.3. A sample of 10 matched healthy controls (HC) were selected from our Laboratory database.
Results: VOI 1. and 2. showed a decrease of N-acetyl-aspartate (NAA), neuronal-axonal marker, and an increase of mio-Inositol (mI), glial marker (NAA/mI, 1.10 and 0.69) compared to HC (2.86±0.61). No significant differences were found for VOI 3. and 4. compared to HC. Interestingly the patient had deficits in visuo- spatial and -constructional abilities related to the WM changes location. At one-year follow-up the DM1 disease showed a relative stability.
Conclusions: The absence in the spectrum of an increase in choline, marker of cell turnover and density, and the absence of lactate and mobile lipids allowed to exclude a brain tumor [4].
This case report highlights that 1H-MRS is a reproducible and sensitive technique for in vivo metabolism evaluation that should be included in the diagnostic work-up of brain focal lesion and in selected cases it may replace the need of biopsy procedure.

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